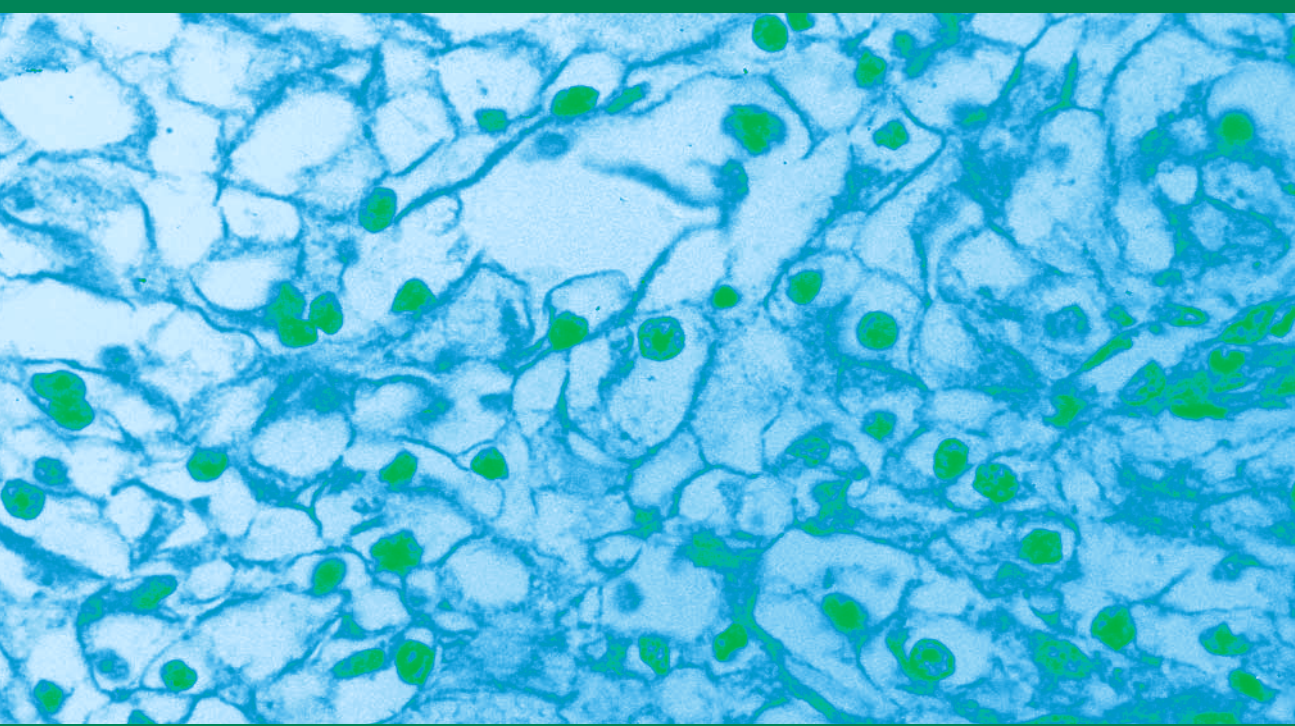
Renal Cell Carcinoma

Molecular Biology, Immunology, and Clinical Management

Edited by
Ronald M. Bukowski, MD
Andrew C. Novick, MD



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PREFACE

Renal cell carcinoma represents a heterogeneous group of tumors, the most common of which is clear cell adenocarcinoma. The annual incidence of this tumor appears to be rising and approximately 12,000 individuals die from this cancer annually in the United States.

One third of patients who present have metastatic disease at the time of diagnosis, and another 40% who undergo nephrectomy will ultimately develop this complication. Over the past 10 years, a significant amount of new information concerning the epidemiology, molecular and immunologic characteristics, and therapy for patients with these tumors has appeared.

The recognition that inherited forms of renal cancer exist, and that chromosomal abnormalities can be identified in these tumors, suggested a genetic basis for renal cell carcinoma. The familial cancer syndrome, Von Hippel Lindau disease, provided the setting in which the genetic abnormalites associated with the development of renal cancer were first described. Abnormalities of the *VHL* gene have also been detected in sporadic clear cell carcinoma, and it has now been recognized that approximately 80 % of these tumors will demonstrate characteristic alterations. Currently the functions of the VHL protein are being investigated, and the biology of clear cell carcinoma of the kidney is under study. Additionally, papillary carcinomas of the kidney appear to express different molecular defects, and these are now being unraveled.

Interest in the immunologic characteristics of renal cancer was based on some of the early observations suggesting spontaneous regression of this tumor and responses to immunologic-based therapy. Recently, it has been recognized that tumor-associated antigens may be present in selected renal cell carcinomas and that recognition of these antigenic structures by the immune system may occur. Additionally, abnormal immune regulation or immune dysfunction has also been described, with the molecular basis of these findings now being studied. The interaction between these two areas may have relevance for the effects of immune-based therapy. The treatment of renal cell carcinoma has also evolved, with improvements in surgical therapy for locally advanced tumors, the introduction of partial nephrectomy, and the recent description of laproscopic techniques for tumor removal. The understanding of the role of these modalities and their use in this patient population is now emerging.

For the majority of patients who have metastatic or advanced renal cell carcinoma that is not surgically curable, therapy remains of limited value. Continued investigation of cytokine-based therapy, adoptive immune strategies, and such newer strategies as the inhibition of angiogenesis is being conducted. Management of these patients often involves surgical removal of metastases and/or residual disease following therapy. Finally, the role of symptom palliation for this patient group is an important issue for individuals with this illness.

Renal Cell Carcinoma: Molecular Biology, Immunology, and Clinical Management was designed to assist physicians and researchers who treat and/or investigate patients with kidney cancer. This volume should assist urologists, medical oncologists, and radiation oncologists in their diagnosis and treatment of renal cell carcinoma. The review is designed to assess the pertinent clinical, biologic, and pathologic characteristics of this illness. New developments in the areas of molecular genetics and immune dysfunction have also been included, focusing on therapy for patients with renal malignancies. The roles of partial nephrectomy, radical nephrectomy, and laparoscopy are covered. Treatment of patients with metastatic disease remains a problematic area, and the modalities that have been used or are being developed are discussed.

The last decade has been a time of innovation in the management of renal cell carcinoma, and we believe that *Renal Cell Carcinoma: Molecular Biology, Immunology, and Clinical Management* will provide an overview of the field, as well as demonstrate the progress that has occurred in this area.

Ronald M. Bukowski, *MD*
Andrew C. Novick, *MD*

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I INTRODUCTION

1 The Epidemiology of Renal Cell Carcinoma

Joshua E. Muscat

CONTENTS

1. INTRODUCTION

The age-adjusted incidence rate of kidney cancer (per 100,000 persons) increased from 6.7 in 1973 to 9.0 in 1992 in the United States *(1)*. Part or all of this increase might be caused by the increased use of modern diagnostic imaging *(2)*. The 1992 rate was 12.6 in men and 6.2 in women; also, rates are slightly higher in black men than in white men. There are no differences in kidney cancer rates between white and black women, and mortality rates of kidney cancer are also similar for whites and blacks. Like most cancers, kidney cancer is uncommon among persons under age 50 yr, but occurs with increasing frequency with older age. The 5-yr survival rate for men and women combined is 57.9%.

With the possible exception of cigarette smoking and high body weight, there are few known causes of kidney cancer. The first reported discovery of a risk factor for kidney cancer came from a 1966 study that examined cause-specific rates of death in over 1,000,000 men and women *(3)*. In cigarette smokers, the rate of kidney cancer was approximately double that in nonsmokers. Because kidney cancer is relatively uncommon, its causes have not been investigated as frequently as lung, breast, and colon cancers. In the past several years, however, there has been an increased effort to examine possible causes of kidney cancer in case-control studies, population-based cohorts, and industrial settings. The largest of these studies, the International Renal Cell Cancer Study (IRCC), included 1732 cases and 2309 controls from Australia, Denmark, Germany, Sweden, and the United States *(4)*. The recent studies have reexamined the association with cigarette smoking, but have also measured the effects of other known human carcinogens or cocarcinogens, such as asbestos and alcohol. In addition, there has been an interest in the role of dietary factors, hormones, and occupational history. Although several environmental and personal exposures have been linked to kidney cancer in these studies, it has proven difficult

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to identify specific causal agents. The current review is not intended to be exhaustive; rather, it examines some conclusions regarding the role of suspected risk factors and explores some of the methodological obstacles in kidney cancer epidemiology.

2. EPIDEMILOGIC FACTORS

2.1. Nutrition

Although a discussion of nutrition is not normally the starting point for a review of kidney cancer risk factors, there are compelling reasons to suggest that diet is the major factor in the causes and prevention of kidney cancer. The role of diet can best be appreciated by comparing international variations in cancer rates. There is an approximately five–eightfold difference between the rates of kidney cancer in Asian populations and Western countries. This international difference exists for many other types of cancer that are likely affected by dietary intake such as breast, colon, and prostate cancer. This fact is often underappreciated by public health researchers. Current research efforts in cancer etiology focus on the identification of genotypes and mutations that are associated with disease. The simple comparison of cancer rates across countries, however, provides important clues to their causes. For example, breast cancer rates are much lower in Japan than in the United States. As Japan has become increasingly Westernized since the 1950s, the rates of breast cancer have increased substantially, although they are still half that in the United States. These observations have provided the basis for numerous dietary studies of mammary carcinogenesis and nutritional epidemiologic analyses. The specific dietary constituents that might affect breast cancer risk have been debated, with dietary fat and soy products hypothesized to play key roles. Table 1 shows the rates of kidney cancer in selected countries *(5)*. The rates are age-adjusted to the world population.

Table 1 shows that the incidence rate of kidney cancer in both men and women is substantially lower in Asian countries than in Western countries. When large differences in cancer rates exist between entire populations, the best explanation for these differences is some factor that is common to most or all of the population. Clearly, diet and nutrition meet this premise. The international differences in kidney cancer rates are not caused by genetics. Mortality rates of kidney and other cancer in first-generation Asian migrants are intermediate between Asian countries and the host Western countries *(6)*. As migrants acquire Western lifestyles, their cancer rates become more similar to Americans and Europeans. In contrast, relatively few individuals in any population are exposed to high levels of industrial chemicals or solvents. Cigarette smoking is another factor that could account for the international variation in rates. Curiously, the prevalence of cigarette smoking is about double in Japanese males than in US males, yet the rates of kidney cancer in Japan is about half that in the United States. This inverse correlation suggests that cigarette smoking does not account for the variations in kidney cancer rates. There is not a perfect correlation between consumption of Western diets and rates of kidney cancer. The rates of kidney cancer in the United Kingdom, for example, are low relative to other European countries. But the table does suggest that diet is likely the most important factor that explains these differences.

Until recently, there have been few epidemiologic investigations of diet and kidney cancer, but several recent studies suggest some intriguing leads. Wolk et al. studied the effects of diet on 698 male cases and 487 female cases diagnosed with renal cell carcinoma (RCC) *(7)*. When comparing dietary history to a control population, statistically sig-

Table 1
International Incidence Rates of Kidney Cancer in Selected Countries, 1983–1986

Country	*Rate for Men*	*Rate for Women*
France, Bas Region	15.21	6.12
Italy, Varese	11.30	5.67
Italy, Tuscany	10.52	5.43
United States. (whites)	10.35	4.86
Italy, Torino	8.55	4.13
France, Isere	6.67	2.79
U.K., England, and Wales	5.55	2.70
Japan, Osaka	4.41	1.81
China, Shanghai	1.98	1.21

nificant observations were observed with total energy intake. There was no association with fat or protein intake independent of energy. Fried meats were associated with increased RCC risk, and protective effects were observed with frequent fruit and vegetable consumption. In a separate analysis, this group reported a significant association with poultry intake, and a decreased risk associated with fruit consumption, especially among nonsmokers *(8)*. Frequent vitamin C and vitamin E consumption was also found to be protective in nonsmokers. In a population-based study in Los Angeles *(9)*, protective effects were found for consumption of cruciferous and dark-green vegetable intake. Protective effects were also observed for carotenoids including beta-carotene. The results of these and other studies were summarized by Wolk et al. *(10)*. They state that the ecologic (international) comparisons that suggest a role for meat and fat intake are not supported by case-control studies. A protective effect of fruits and vegetables has been observed in the majority of studies that have examined diet as a risk factor for kidney cancer. They conclude that whereas diet is likely a factor in the etiology of RCC, contradictory results and methodological limitations in case-control studies prevent definite conclusions. These conclusions are for the most part reasonable, if not somewhat discouraging.

A more optimistic viewpoint maintains that diet is an important, if the not the most important, factor in the development of RCC. Added to the problem of homogeneity in the diet, responses to questions on food frequency are not very reliable. Because dietary habits may change over time, and/or the nutritional composition of food may change with time or vary by geography, questions on food frequency are relatively poor predictors of cancer. There may be complex combinations of dietary factors that affect cancer risk, but cannot be measured by food-frequency questionnaires. One large-scale cohort study that may shed more light on the role of diet and kidney cancer is the Adventist Mortality Study. The dietary habits of Seventh Day Adventist men, of whom approximately one-half are exclusively lacto-ovovegetarian (consuming meat, poultry, and fish less than one time per week) *(11)*, have been examined in relation to breast, prostate, and colon cancer. Because there is a wider degree of dietary variability within this cohort than what would be found in other population groups, the effects of specific dietary constituents such as meat and protein intake in relation to kidney cancer can be examined with higher precision. Further, most Adventists abstain from tobacco products and alcohol consumption. Because smokers consume more alcohol and meat, and less fruits and vegetables than nonsmokers *(12)*, the confounding effect that cigarette smoking has on dietary estimates

of risk are minimized in the Adventist study. If diet has an association with kidney cancer only in smokers, than other populations might be more suitable as study subjects.

Laboratory model studies of diet and cancer are expensive and involve large numbers of animals to assess the effects of a single dietary constituent. It is often prohibitively expensive to examine the effects of dietary "cocktails" in carcinogenesis studies. Similarly, randomized clinical trials of dietary interventions often require large numbers of study subjects. Perhaps the most publicized examples of the difficulties in assessing the role of diet in cancer were two trials of dietary supplements and lung cancer risk. It has been hypothesized for more than two decades that beta-carotene and vitamin A might inhibit the occurrence of lung cancer. These hypotheses were based on epidemiologic observations, findings from animal experiments, and the biological properties of these antioxidants. In 1996, Omenn et al. published the findings from the Beta Carotene and Retinol Efficacy Trial (CARET) that included 18,314 smokers and former smokers *(13)*. The treatment arm received 25,000 international units (IU) of retinal and a 30 mg beta-carotene supplement per day. The trial ceased when preliminary findings showed a significantly increased risk of lung cancer in the experimental group at 21 mo. In another randomized trial of beta-carotene that enrolled healthy men only, 12 years of beta-carotene supplementation did not lower the incidence of any type of cancer *(14)*. These unexpected findings underscore the complex, and often contradictory, nature of dietary research. It might not be feasible to adopt an Asian diet in Western countries simply because of the unavailability of certain foods. However, the identification of specific foods common in Western diets that increase the risk of kidney cancer is a long-term goal that has preventive implications.

2.2. Cigarette Smoking

Cigarette smoking has been linked to an increased risk of kidney cancer although the effects are moderate *(15–20)*. Increased risks have most often been found in long-term or heavy smokers. In these groups, the magnitude of relative risks have ranged from approx 1.5–2.0 across studies. Although it has been concluded in other authoritative texts that there is a causal role of cigarette smoking in the etiology of kidney cancer *(21,22)*, the scientific data that support this contention is not overwhelming. Several studies of kidney cancer found no association with cigarette smoking *(23–28)*. One case-control study found an effect only in men and no relationship with the number of cigarettes smoked per day *(14)*. Another study showed an association only in current smokers *(29)*.

Although renal tumors have been experimentally induced in animals exposed to chemical carcinogens, cigarette carcinogens do not cause kidney cancer in animals. The two known major classes of tobacco carcinogens are the polycyclic aromatic hydrocarbons (PAH) such as benzo[a]pyrene and the tobacco-specific nitrosamines. The most well-characterized nitrosamines are 4-(methylnitrosamino)-1-(3-pyridyl)-1-(butanone) (NNK), which induce lung tumors in animal studies, and N-nitrosonornicotine (NNN), which are oral cavity carcinogens. Exposures to nitrosamines also occur in the diet and in occupational settings. PAH and tobacco-specific nitrosamines do not produce kidney tumors in experimental animals. There might be other compounds within tobacco smoke that cause kidney tumors in animals, such as heavy metals, but none have been identified.

It is unlikely that the epidemiologic findings showing a relationship between cigarette smoking and kidney cancer are an artifact. Although cigarette smoking is linked with other adverse lifestyle behaviors, such as alcohol consumption and a diet low in fruits and

vegetables, these other risk factors are only modestly associated with kidney cancer in epidemiologic studies. It has been demonstrated empirically that a confounder (e.g. diet, alcohol, and so on) must have a strong relationship with a study end point to cause a spurious association with the risk factor of interest (e.g., smoking). Among studies that do show an association with cigarette smoking, the findings generally show a dose-response gradient. Smoking cessation is currently the best means to reducing kidney cancer incidence.

Some dietary approaches to chemoprevention of cancer are based on the identification of specific carcinogens and compounds that inhibit their tumorigenic properties. There are a large number of compounds that inhibit B[a]P and NNK-induced lung cancers in animals including phenyl isothiocyanate (PEITC) and benzyl isothiocyanate *(30)*. PEITC occurs in high concentrations in cruciferous vegetables, and human studies of volunteer smokers who consumed watercress demonstrate reduced metabolic activation of NNK *(31)*. These studies suggest that if cigarette smoke is a cause of kidney cancer, the identification of specific kidney carcinogens in tobacco smoke might lead to dietary intervention studies in smokers. However, the failure to reduce lung cancer rates in the CARET study shows the difficulty in identifying chemopreventive agents, their appropriate dosages, and timing of administration. It is unlikely that dietary interventions will be conducted to inhibit kidney cancer in the near future as public health priorities focus on the more prevalent cancers.

2.3. Alcohol

The national geographic variation in the rates of kidney cancer mortality is correlated with per capita intake of alcohol *(32,33)*. These findings have not been corroborated in the majority of case-control studies or in cohort studies *(34–37)*. The mechanisms for alcohol carcinogenesis are poorly understood. Epidemiologic data and experimental studies in animals suggest that alcohol is a cocarcinogen with tobacco by apparently modifying the effects of tobacco carcinogens in specific organs such as the oropharynx, larynx, esophagus, and liver, but not lung. If alcohol is a cocarcinogen for kidney cancer, it is not possible to detect its effects because of the weak carcinogenic action of cigarette smoking.

2.4. Coffee

The international geographic variation in kidney cancer mortality rate is correlated with per capita coffee consumption *(38,39)*. These observations have not been verified in case-control studies after statistical adjustment for cigarette smoking. In prospective studies, coffee consumption did not increase the risk of any type of cancer *(40,41)*.

2.5. Occupation

There have been several studies of occupation or industrial exposures and kidney cancer risk. A statistically increased rate of cancer was observed in a cohort of German cardboard factory workers exposed to trichloroethene (TCE) *(42)*. Five cases of kidney cancer were observed, compared to two cases from a cancer registry. These data were unadjusted for cigarette smoking. In a follow-up case-control study in Germany that included 58 patients with RCC, 19 had a history of exposure to TCE, compared to 5 of 84 controls *(43)*. These findings seem somewhat suspect, however. It seems highly unlikely that one-third of all kidney cancer patients in a general hospital would have a common exposure to a specific industrial chemical. In a case-control study of 45 kidney cancer

cases with a history of exposure to TCE and a similar number of controls also exposed to TCE, there was increased risk for subjects possessing the *GSTM1* and *GSTT1* polymorphisms *(44)*. However, this finding does not implicate TCE as a risk factor. The mortality rate of kidney cancer was calculated for 879 men and women employed in the manufacturing of hydroquinone from 1942–1990 *(45)*. No excess rate of kidney cancer was found. In a cohort study of Italian oil refinery workers, elevated mortality rates of kidney cancer were found for maintenance workers, although only two cases were detected and zero expected *(46)*. An elevated rate of kidney cancer was found in a petrochemical refinery, but this was not related to exposure to various manufacturing processes *(47)*. A proportionate mortality study of 13,301 construction iron workers found an increased risk for all cancers, but not kidney cancer specifically *(48)*. Another proportionate mortality study of 1553 Spanish steel workers employed from 1986 to 1993, found a 1.89 increased risk of kidney cancer *(49)*. Using a population-based registry, Lowery et al. found an increased risk of kidney cancer for architects, but not for other professional groups *(50)*.

In the IRCC study of 1732 kidney cancer cases, significant associations were found with employment in blast furnaces, the coke oven industry, the iron and steel industry, and exposure to asbestos, cadmium, dry-cleaning solvents, gasoline, and other petroleum products *(51)*. A report from New Zealand-based cases in the New Zealand Cancer Registry found significantly increased risks of RCC in firefighters and painters *(52)*. In a German case-control study of 277 cases of RCC, elevated risks were found for employment in metal-related industries and exposure to perchloroethlyene and tetrachlorocarbonate *(53)*. No other type of occupational exposure or employment was related to kidney cancer risk. In a Danish population-based control study of 365 cases, increased risks were found in truck drivers, and workers exposed to gasoline, other hydrocarbons and insecticides/herbicides *(54)*. A Finnish study of 338 case-control pairs found elevated risks for white-collar occupations, employment in the chemical and printing industry, manufacturing of metal products, iron, and metal work and mail, telephone, and telegraph jobs *(55)*. No increased risk of kidney cancer was found at a Texas chemical manufacturing plant after 49 years of follow-up, although exposures to specific chemicals was not studied *(56)*. In a French study of 196 cases, increased risks were found for sales workers, managers, textile workers, and tailors *(57)*.

Reviews of studies of exposure to gasoline *(58)*, asbestos *(59)*, and inorganic lead compounds *(60)* conclude that these exposures are unrelated to kidney cancer. Similarly, although isolated findings of increased risks associated with other occupations or occupational exposures have been reported in other studies, there are no specific occupational carcinogens that have been consistently linked to kidney cancer.

2.6. Hormonal Factors

The IRCC Study *(61)* found that the risk of kidney cancer in women increased linearly with two or more births, compared to one birth. Unrelated factors were age at menarche and menopause, and estrogen replacement therapy. Oral contraceptive use in nonsmoking women was associated with a decreased risk. A twofold increase associated with 5 or more births was observed in another population-based case-control study *(62)*. In other population-based studies, the risk associated with parity was either low *(63)* or none *(64,65)*. These and other studies *(66,67)* also show no effect of exogenous estrogen use. It is unclear how high parity might increase the risk of kidney cancer.

2.7. High Body Weight

Body mass index or high body weight has been consistently linked to kidney cancer in women. In men, the positive associations have been observed as well, but the findings are inconsistent or weak. The proposed mechanism that explains this observation is that obesity increases estrogen production. It is well established that estrogens promote the development of kidney tumors in hamsters *(68)*. Whether obesity itself is a risk factor or some factor associated with obesity remains unclear. More data are also needed to determine whether life-long obesity or obesity in adulthood increases the risk. In addition, the most appropriate anthropometric measurement for classifying excess body weight has been debated and depends on the study outcome *(69)*. Abdominal obesity, waist–hip ratio, and other measures have been used as measurement tools to study the health effects of excess weight. Weight gain or cyclical weight loss or adiposity has not been examined in relation to kidney cancer risk in detail. Whether such measures are appropriate predictors of kidney cancer risk needs to be determined. One recent finding from a cohort study in women found that various measures of weight and adiposity were risk factors *(70)*.

The finding of excess weight as a risk factor in men and the inconsistent findings associated with reproductive factors in women raises alternative hypothesis to the hormonal one. One possibility is that high caloric intake, and not estrogens, might be the underlying mechanism for the above findings.

2.8. Treatment of High Blood Pressure

Along with obesity and cigarette smoking, high blood pressure or treatment for high blood pressure has been linked to kidney cancer risk in a number of studies. In a cohort of 8006 men living in Hawaii, there was no association between hypertension and kidney cancer incidence after adjusting for the use of antihypertensive medication *(71)*. Other studies find that hypertension in nonmedicated persons does *(65)* and does not increase risk of kidney cancer *(72)*. Calcium channel blockers have been linked to an increased overall mortality from cancer in some studies (reviewed by Straka and Swanson *[73]*, and Cheng and Behar *[74]*) but not in other studies *(75–78)*. Calcium channel blockers inhibit cell differentiation and apoptosis. In a cohort study of 18,635 US nurses, self-reported use of calcium channel blockers was unrelated to the incidence of kidney cancer *(79)*. In contrast, a multicenter case-control study of incident cancer showed a significant 1.9-fold increased risk of death from kidney cancer in users of calcium channel blockers. The same study found a significant 1.8-fold risk of kidney cancer associated with beta-blockers. Angiostensis converting enzyme (ACE) inhibitors have been associated with both increases in risk *(80)*, no changes in risk *(81)*, and decreased risk *(76)*.

Diuretic use has been shown to increase kidney cancer risk in a number of studies. In experimental studies, diuretic exposure induces tubular adenocarcinoma. Similar to the results from studies of other antihypertensive agents, the epidemiologic findings are conflicting and marked with ambiguity. Positive associations with diuretic use and not hypertension were reported in a cohort of women *(70)*. In the IRCC Study and two other population-based case-control studies, it was reported that the independent effects of treatment for hypertension could not be distinguished from the effects of hypertension *(82–84)*. Similarly, obesity or other unmeasured factors might have confounded increased risks associated with diuretics in two cohort studies *(85,86)*. Other population-based case-control studies have reported no association with diuretics *(87)* or only in women

(29). The above medications are commonly prescribed to treat hypertension, and significant challenges face epidemiologists in teasing out a causal agent from a complex constellation of possible risk factors.

3. PREVENTION

Interventions to reduce the occurrence of kidney cancer should focus on smoking cessation/prevention. Weight loss might also reduce the risk of kidney cancer although target weights need to be developed. There is less data implicating excess weight as a risk factor in men, and weight loss in men cannot be currently recommended as a prevention strategy. It is noteworthy that low body mass increases the risk of certain cancers including larynx *(88)* and lung *(89)* and that weight loss may only be advisable in combination with smoking cessation. Because smoking cessation is often accompanied by weight gain, this may prove difficult for some smokers. Of course, weight loss might be beneficial for other health reasons and the various pros and cons need to be considered. Proper industrial hygiene is necessary to minimize exposure to hazards in the work environment, but there are no specific occupational exposures that have been definitively identified as renal carcinogens. The international differences in kidney cancer incidence has not been explained by other factors and diet remains the most compelling reason for these differences. At present, there is insufficient information to make specific dietary recommendations in smokers. Because there are no other high-risk groups for kidney cancer, it might be prudent to follow dietary recommendations that are suggested to reduce the overall risk of mortality from coronary artery disease and cancer. This would include the frequent consumption of grains, fruits, and vegetables, and minimizing fat and calorie intake. One suggestion for the optimum diet is 25% of calories from fat and 25 grams per day of fiber *(90)*.

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2 The Pathology of Renal Neoplasms

Howard S. Levin and Jonathan L. Myles

CONTENTS

1. RENAL CELL CARCINOMA

The classification of renal cell carcinoma is undergoing an evolutionary process. At one time, most renal cell carcinomas were classified as clear cell type, granular cell type, or sarcomatoid type. Although this antiquated classification scheme has provided a framework for classification and prognosis, more modern concepts of subtyping renal cell carcinoma have evolved based on histogenesis. Renal cell carcinomas are currently classified as representing clear cell, papillary (chromophil), chromophobe, collecting duct, and unclassified types, which include the pure sarcomatoid variant *(1)*.

The most common subtype of renal cell carcinoma is the clear cell variant. Clear cell carcinoma of the kidney accounts for 70% of primary renal neoplasms. Grossly, the neoplasms have a characteristic tan-yellow appearance. Foci of hemorrhage and necrosis are evident (Figs. 1 and 2). The neoplasms tend to invade the adjacent renal tissue in an infiltrative manner (Fig. 3). Often adjacent to the main tumor mass are small satellite lesions. Gross examination of the kidney should include an evaluation for the presence of capsular invasion. Often, it is easier to determine if capsular invasion is present by examining the gross specimen. Histologically, the infiltrating border may have a fibrous

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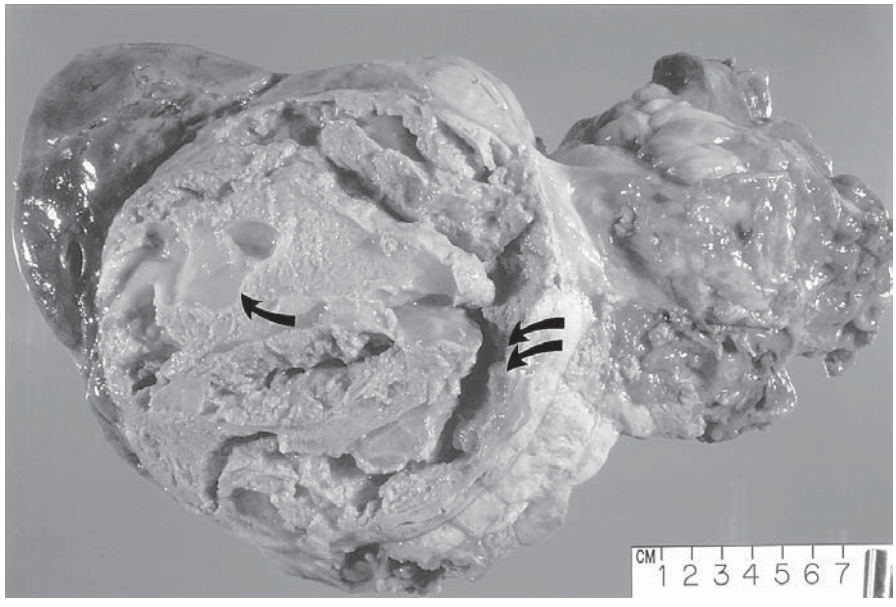

Fig. 1. Renal cell carcinoma. Grossly, the neoplasms have a spherical shape. Cystic change may be evident (arrow) and foci of necrosis are common (discohesive areas, double arrow).

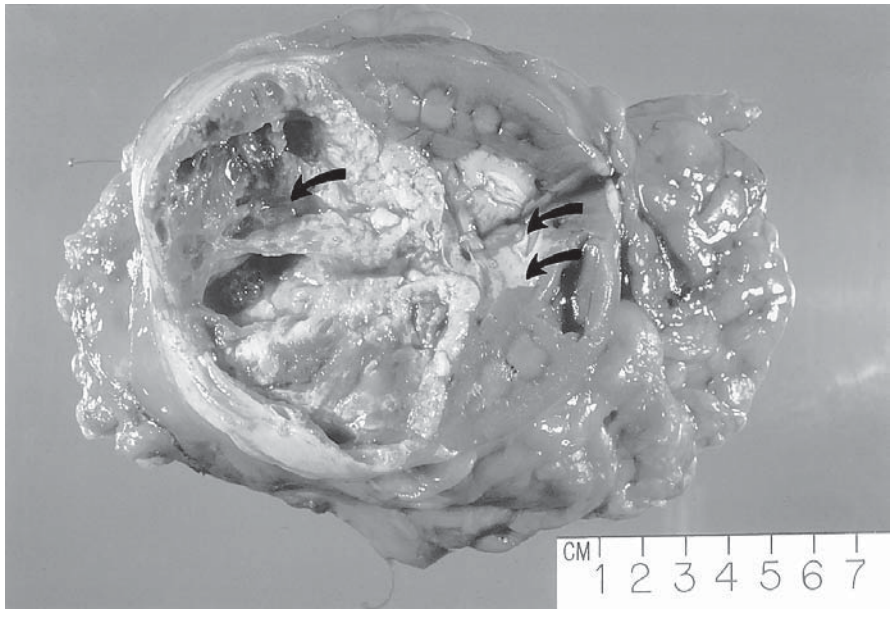

Fig. 2. Hemorrhage and necrosis in renal cell carcinoma (arrow). Satellite nodules may be present (double arrow).

rim, which simulates a true renal capsule. Gross examination also should include evaluation of the renal sinus, the area immediately adjacent to the vessels and ureter in the hilum. Invasion into the renal sinus fat or perinephric fat places the neoplasm in the T3 category *(2)*.

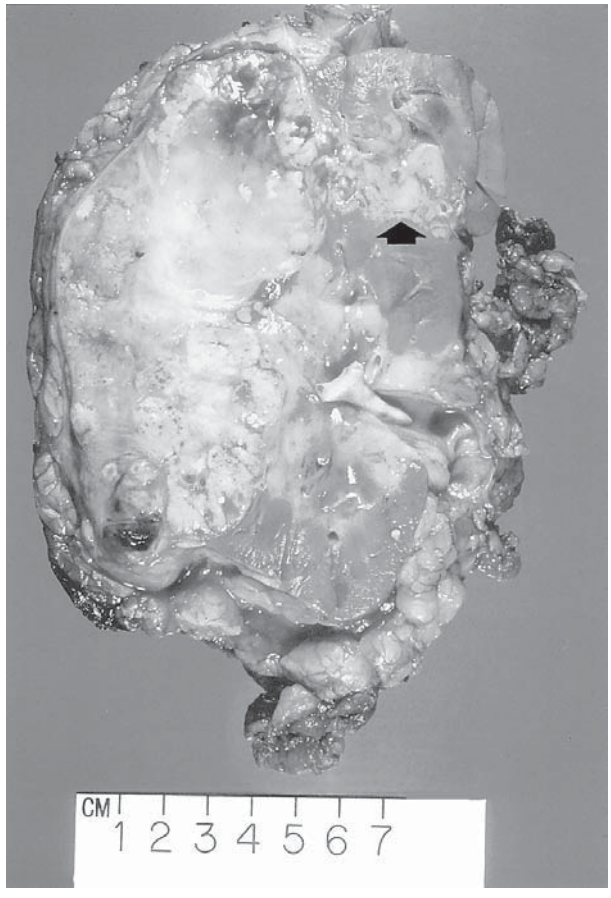

Fig. 3. Renal cell carcinoma. The neoplasm is invading the adjacent renal parenchyma in an infiltrative manner (arrow).

Microscopically, the neoplastic cells have a characteristic clear cytoplasm, although foci of cells with eosinophilic cytoplasm are common. In some clear subtypes, the predominant cytoplasmic staining pattern is eosinophilic. A characteristic feature of clear cell carcinoma is a delicate branching vasculature (Fig. 4). This feature is most useful in evaluating clear cell carcinomas present in metastatic sites. The presence of a delicate branching vasculature in other sites may provide a clue that the kidney is the origin of the neoplasm. However, this feature is not specific for renal origin.

In prior classification schemes, the second most common subtype of renal cell carcinoma was the granular cell variant. In that histologic type, the neoplastic cells had an eosinophilic cytoplasm. The granular cell variant of renal cell carcinoma is not recognized in current classification schemes. If lesions have the characteristic nested appearance of the clear cell subtype and a portion of the cells have clear cytoplasm, the lesion is classified as representing the clear cell subtype. These neoplasms also characteristically have a delicate branching vasculature. Neoplasms previously classified as the granular cell variant of renal cell carcinoma are most likely classified now as representing oncocytomas, chromophobe carcinomas, papillary carcinomas, collecting duct carcinomas, or epithelioid angiomyolipomas.

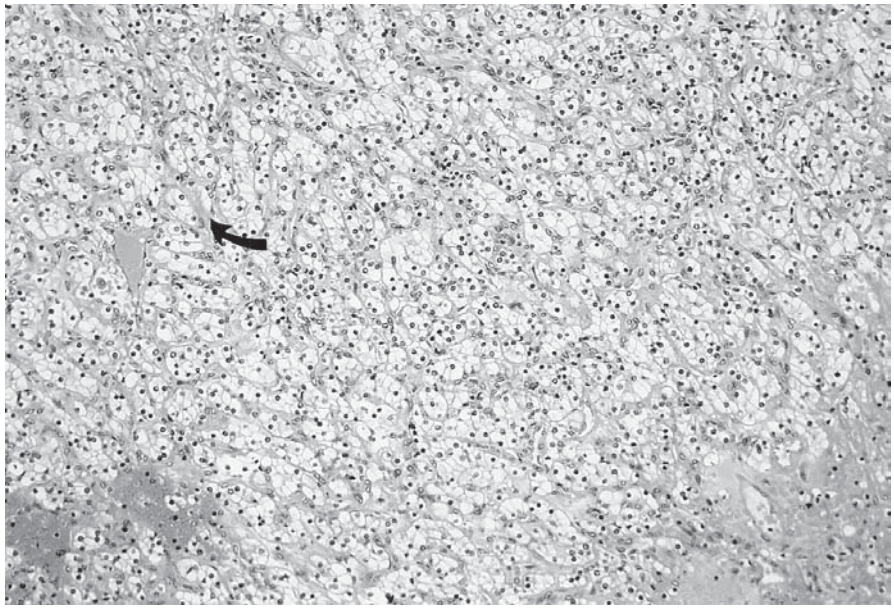

Fig. 4. Clear cell carcinoma. The neoplastic cells have a characteristic clear cytoplasm and a delicate finely branching vasculature (arrow). H&E x100.

Papillary renal cell carcinoma is also known as the chromophil subtype of renal cell carcinoma. It is the second most common subtype of renal cell carcinoma and represents approximately 15% of all cases. The gross appearance is similar to that of a clear cell carcinoma, although the neoplasm tends to be not as bright and has a brownish hue. The histologic features are characteristic for this subtype. A papillary architecture is present. There will be thin fibrovascular cores lined by neoplastic cells. The neoplastic cells may either have a granular or clear cytoplasm. The proportion of the neoplasm that has a papillary morphologic appearance will vary from tumor to tumor. Psammoma bodies are commonly seen in papillary renal cell carcinoma and calcification may be noted grossly. Another characteristic feature is the presence of foamy macrophages in the papillary fronds (Fig. 5).

Chromophobe renal cell carcinoma is becoming an increasingly recognized entity. These carcinomas represent approximately 5% of all renal carcinomas. Within a given region of the neoplasm, the nuclei tend to vary in size more so than other renal carcinomas. There is often a clear halo present within the neoplastic cells (Fig. 6). There is condensation of the cytoplasm near the cell membrane to give this perinuclear halo. The cell membranes often appear thickened, resembling plant cell walls. The neoplastic cells tend to aggregate in small solid nests. Clear cells, as well as granular cells, are typically present in the neoplasm. A characteristic feature is the presence of strong blue staining with colloidal iron stain in the cytoplasm of the neoplastic cells. By electron microscopy, microvesicle structures are present in the perinuclear region (Fig. 7) *(3)*.

Immunohistochemical stains may be useful in identifying chromophobe renal cell carcinomas *(4)*. Chromophobe renal cell carcinomas typically stain positive for E-cadherin and negative for N-cadherin. Typical clear cell carcinoma stains negative for E-cadherin and positive for N-cadherin. The distinction of chromophobe carcinoma from oncocytoma needs to be made using conventional light microscopic criteria.

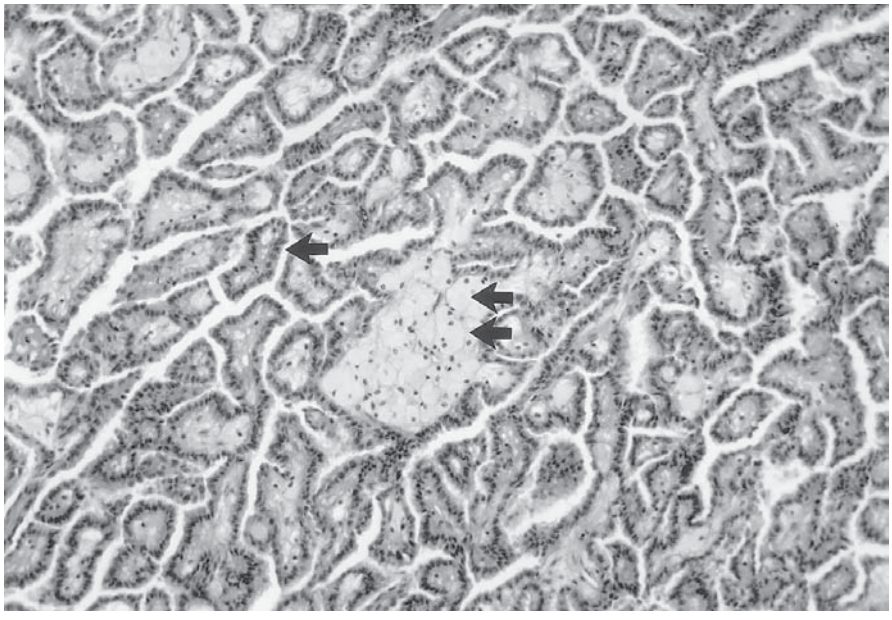

Fig. 5. Papillary renal cell carcinoma. The neoplastic cells line papillary fronds (arrow). Foamy macrophages may be present in some papillary fronds (double arrow). H&E x200.

Pure sarcomatoid renal cell carcinomas are rare. If a renal carcinoma is of the pure sarcomatoid type, in the current classification system it is listed in the renal cell carcinoma, unclassified category. Typically, tumors that demonstrate sarcomatoid differentiation will demonstrate another recognized variant of renal cell carcinoma if multiple sections are obtained (Fig. 8). The tumor is categorized as representing the recognizable subtype, with the modifier that sarcomatoid areas are evident. Unclassified renal cell carcinoma represents approximately 4–5% of cases. These include composite tumors and tumors that produce mucin in addition to pure sarcomatoid tumors.

Prognostic factors in renal cell carcinoma are divided into two main types: patient related and tumor related *(5,6)*. The following features are unfavorable patient related prognostic factors: weight loss greater than 10%, ESR greater than 30 anemia, increased serum calcium, and increased alkaline phosphastase. Unfavorable tumor-related prognostic factors include positive surgical margins, multiple metastatic lesions, metastatic lesions to the liver or lung, unresectable metastatic lesions, high nuclear grade, clear cell or collecting duct carcinoma, sarcomatoid change, and DNA aneuploidy.

Nuclear grade has been shown by multiple studies to be an easily recognizable prognostic indicator, however, studies fail to agree on where break points should occur in grading, particularly in the midpoints of the grading scheme *(7)*. The most widely used nuclear grading system is that of the Fuhrman nuclear grade *(8)*. Fuhrman criteria are shown in Table 1:

Table 1
Fuhrman Criteria

Grade I—Inconspicuous or absent nucleoli, 10 micron nuclei
Grade II—Nucleoli at 400x, 15 micron nuclei
Grade III—Nucleoli at 100x, 20 micron nuclei
Grade IV—Bizarre multilobate nuclei with heavy chromatin clumps

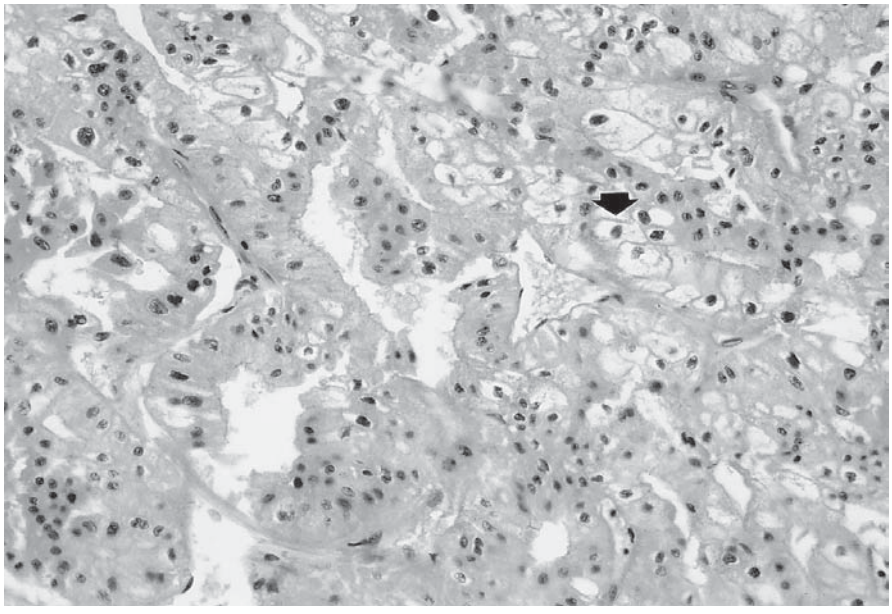

Fig. 6. Chromophobe renal cell carcinoma. The neoplasms often have areas of cells with clear cytoplasm as well as eosinophilic cytoplasm. Cell membranes are prominent (arrow). H&E x400.

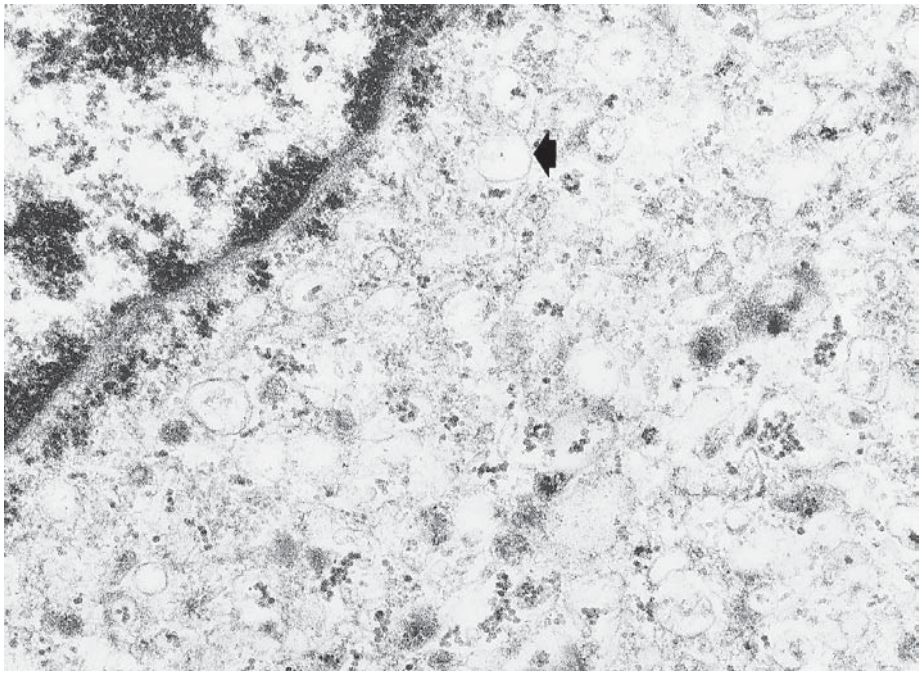

Fig. 7. Electron micrograph of chromophobe renal cell carcinoma. Microvesicles (arrow) are present in a perinuclear location (x24,000).

Patients with grade I nuclei have a relatively lower risk of distant metastasis when compared to patients with grade IV nuclei. The prognosis for patients with grade II or III nuclei are intermediate. Although there is some disagreement in the literature as to where

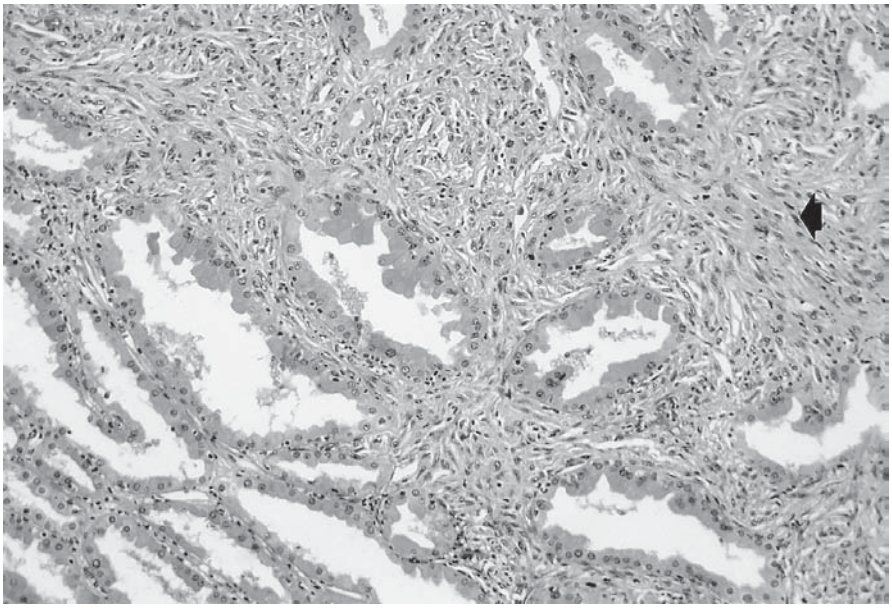

Fig. 8. "Sarcomatoid" differentiation (arrow) adjacent to an area of "clear cell" differentiation with eosinophilic cytoplasm in renal cell carcinoma. H&E x200.

favorable and unfavorable break points occur in the Fuhrman nuclear grade, it is generally agreed that most papillary tumors that metastasize are of high nuclear grade and also that low-grade clear cell carcinoma and chromophobe carcinoma may metastasize. The most common nuclear grade using Fuhrman criteria is grade II. Patients with sarcomatoid differentiation have a very poor prognosis, whereas chromophobe carcinomas tend to have a good prognosis. The prognosis for papillary carcinoma is better than clear cell carcinoma in low-stage and low-grade neoplasms.

The primary location of metastatic lesions varies according to subtype *(9)*. The most common location of metastasis with clear cell carcinoma is the lung. Papillary types most commonly metastasize to lymph nodes. Chromophobe types most commonly metastasize to the liver. Sarcomatoid subtypes metastasize to various locations.

Size is also a useful prognostic indicator. Metastatic lesions are unusual if the neoplasm is less than 3 cm in diameter. Metastasis is quite common in lesions greater than 12 cm in size.

The staging of renal cell epithelial neoplasms has been discussed in detail elsewhere in this text. There are several gross and histologic features of the neoplasm to which the pathologist needs to pay particular attention regarding staging of the neoplasm. Tumors measuring less than 7 cm in diameter that are limited to the kidney are classified as T1 tumors. T2 tumors are greater than 7 cm, but are still limited to the kidney. Invasion of the renal vein, adrenal gland, or perinephric fat places the tumor in the T3 category. Extension of the neoplasm into the adrenal gland generally represents local extension, rather than metastasis. Invasion into the renal sinus fat places the tumor in the T3 category. We routinely examine the renal sinus fat histologically to evaluate for the presence of neoplasm. If the tumor invades beyond Gerota's fascia, it is classified as T4.

Renal cell adenomas are currently defined as lesions measuring less than 5 mm in size with a low-grade tubulopapillary morphology (Fig. 9) *(10)*. High nuclear grade lesions should be

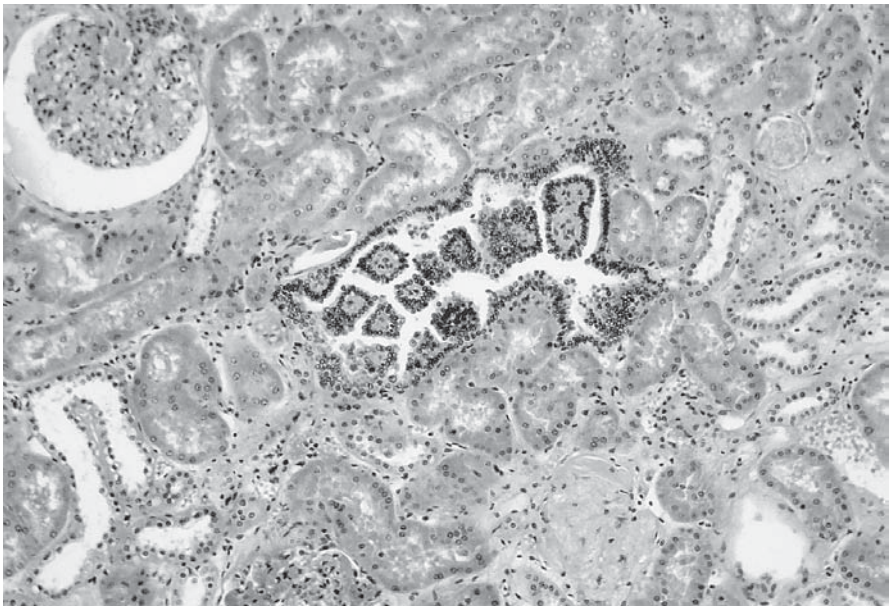

Fig. 9. Renal cell adenoma. The lesion measures less than 5 mm in size and contains a low-grade tubulopapillary morphology. H&E x200.

classified as carcinoma, regardless of size. If the neoplasm contains clear cells in a solid or in a tubulopapillary pattern, the lesion should be classified as carcinoma.

Renal cell carcinoma must also be distinguished from benign renal cysts. A cyst lined by a layer or two of clear cells should be assessed conservatively if the nuclei do not appear anaplastic. Nodular proliferations of clear cells in the cyst or mural invasion are indicative of carcinoma (Fig. 10).

2. RENAL MEDULLARY CARCINOMA

Renal medullary carcinoma (RMC) is a highly malignant adenocarcinoma existing predominantly in African-Americans. Less than 100 cases had been reported by 1998. The reported patients are young in age, ranging between 6 and 39 years old, and most have sickle cell trait. Abrahams et al's 28 cases with a mean age of 13 years from the National Wilms' Tumor Study indicate that cases may develop early in childhood *(11)*. Almost all cases had extrarenal involvement at the time of nephrectomy. We have studied one patient who underwent partial nephrectomy. Although there was no evidence of extrarenal involvement at the time of surgery, metastases were clinically apparent within several months after surgery. Most patients have died within 1 yr of diagnosis. The mean duration of survival in the original report was 15 wk *(12)*.

The main tumor mass is generally in the renal medulla and ranges from 4 to 12 cm in maximum dimension (Fig. 11). Satellite tumors are often present in the cortex, pelvis, or perirenal blood vessels. The tumors are generally variegated in appearance.

RMCs have a variable morphology (Fig. 12). Under low-power magnification, they form both macrocystic and microcystic spaces, sometimes resembling yolk sac tumor, as well as solid sheets. Some formations resemble adenoid cystic carcinoma. Most tumors have poorly differentiated, sometimes spindled areas with edematous and desmoplastic stromal changes, a neutrophilic reaction and extensive necrosis. Lymphatic invasion and

Fig. 10. Cystic renal cell carcinoma. The clear cells lining the cyst (arrow) are present in multiple layers. H&E x200.

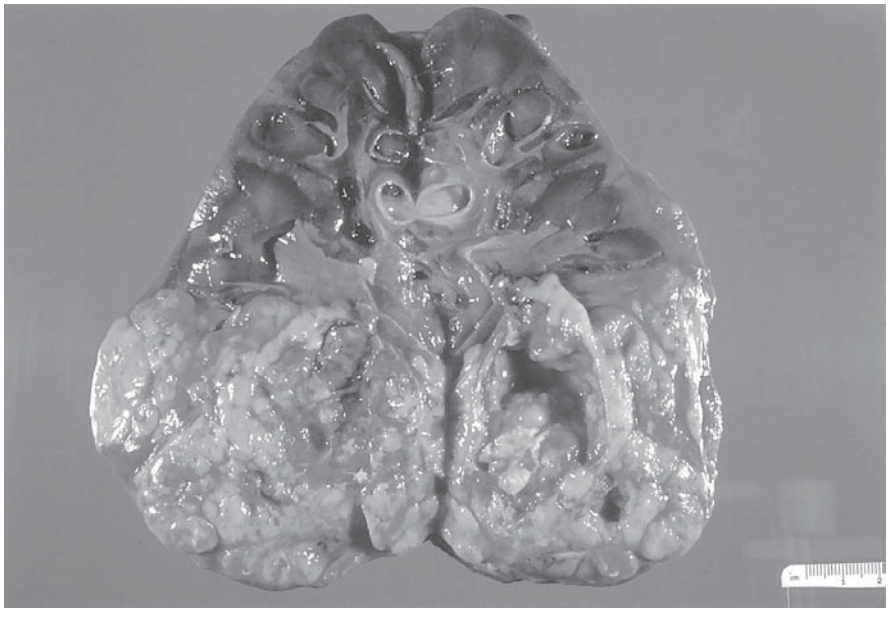

Fig. 11. Renal medullary carcinoma. Bivalved kidney with solid variegated yellow-tan 12-cm tumor involving collecting system, sinus, medulla, and cortex.

vascular invasion are common. The individual tumor cells usually have vesicular, variably shaped nuclei and prominent nucleoli with eosinophilic to dark cytoplasm. Rhabdoid and plasmacytoid forms have been reported. In the original report of 34 cases, all had foci of sickle cells *(12)*. A recent report from the National Wilms' Tumor Study found 28 cases in

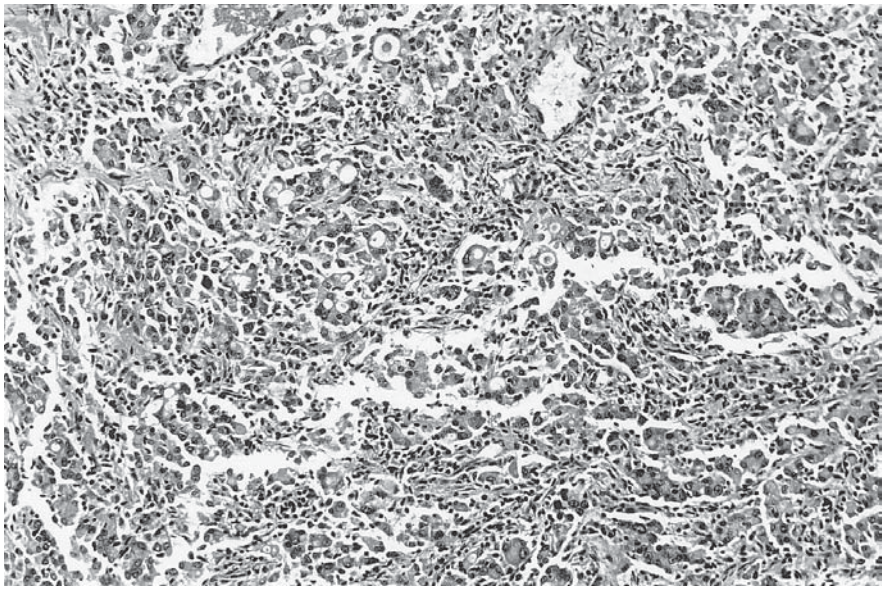

Fig. 12. Renal medullary carcinoma. Poorly differentiated carcinoma with focal glandular differentiation and rare vacuolated cells. 27-yr-old African-American female with sickle cell trait. H&E x130.

17 years, 26 of which had sickle cells *(11)*. Immunohistochemical stains have demonstrated keratin positivity in most cases and CEA positivity in some. Abrahams et al. described positivity for keratin (CAM 5.2), vimentin, and EMA. Some tumors demonstrate cytoplasmic mucin with PAS and mucicarmine stains *(11)*.

Metastases are usually present in lymph nodes and have been found in adrenal gland, liver, and lungs. Perirenal tissues including blood vessels, renal sinus, and retroperitoneum are usually involved by tumor.

Cytogenetic studies have revealed abnormalities of chromosome 3, and four cases demonstrated monosomy of chromosome 3 *(13)*.

3. METANEPHRIC ADENOMA

Metanephric adenoma (MA) is a benign renal neoplasm that may be mistaken for renal cell carcinoma or epithelioid nephroblastoma. These tumors occur predominantly in women. In the two largest series of 50 and 7 cases, the age range was 5–83 yr with medians of 41 and 49 yr *(14,15)*. In the two series, there were 42 females and 15 males. Clinical features are usually nonspecific, but patients have presented with hypertension, hematuria, polycythemia, fever, and palpable masses. None of the tumors have recurred or metastasized after surgery. However, four patients had associated low-grade renal cell carcinomas from 0.8 to 4 cm in diameter *(14,15)*.

The tumors reported have been mostly solitary, not bilateral, and ranged from 0.3 to 15 cm in maximum dimension with a mean diameter of approx 5 cm (Fig. 13). They have occurred approximately equally in both kidneys and occurred in all parts of the kidneys. Approximately 20 MAs had gross calcification with an occasional tumor totally calcified. Generally, capsules were inapparent or partial. On cut section, the tumors varied from gray to tan to yellow and often contained hemorrhagic and necrotic areas. Some tumors were partially cystic *(14,15)*.

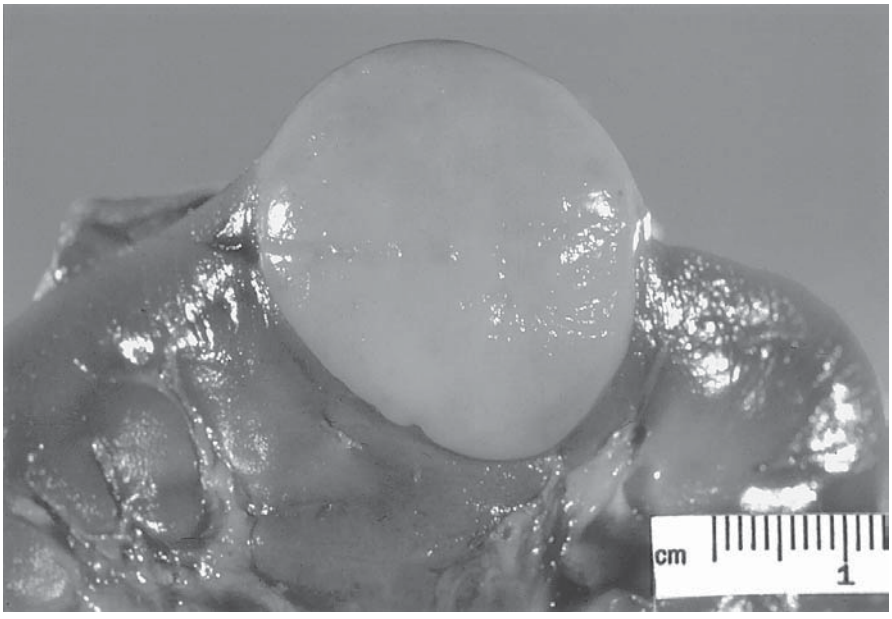

Fig. 13. Metanephric adenoma. 3.5-cm discrete unencapsulated tan nodule that bulges the renal capsule.

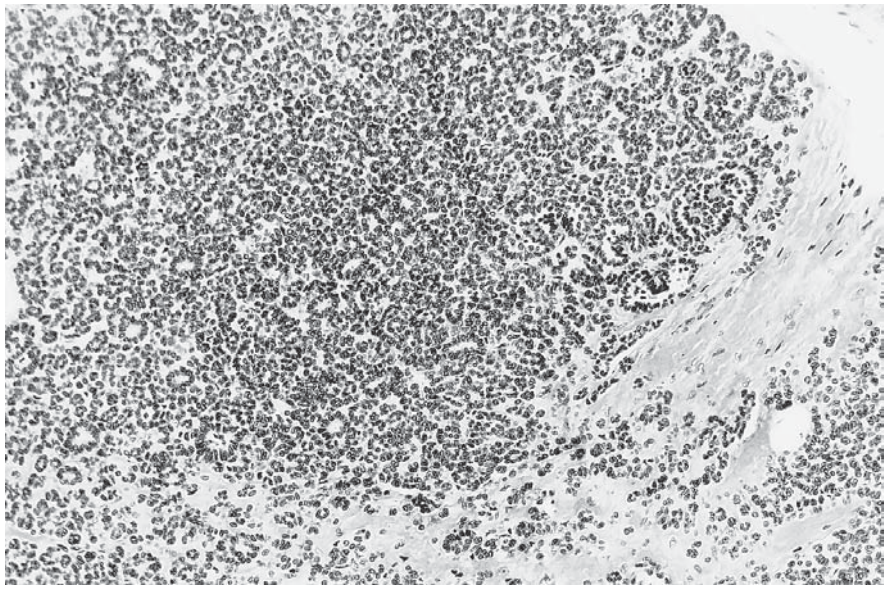

Fig. 14. Metanephric adenoma comprised of tubular and focally papillary structures with little stroma. The nuclei are small and dark with little cytoplasm. H&E x130.

MAs are comprised of small acini in an acellular stroma (Fig. 14). Sometimes acini are in loose or hyalinized connective tissue. Other times, acini are closely packed and have a solid appearance. Papillary, somewhat glomeruloid, structures are common. The individual epithelial cells of the acini are small and round to ovoid. Nuclei are small and inconspicuous. Some nuclei are clefted. Tubules are often lined by a basement membrane. Psammoma bodies may be present. Rare microcystic and blastema-like formations

were described by Davis et al. *(14)*. Mitotic figures are rare and not atypical. There is absence of metanephric blastema.

Immunohistochemical stains demonstrated that acinar cells were generally positive for keratin and generally negative for EMA. Tubular structures have stained positively with peanut lectin. This is consistent with distal nephron and tubule differentiation *(14)*. Jones et al. described three of five cases immunoreactive for *Leu-7* and one of six cases immunoreactive for EMA and muscle-specific actin *(15)*.

The presence of scarring and calcification in some cases has suggested that individual MAs have been present over an extended period of time. Davis described a 5-yr history of polycythemia prior to surgery together with persistent X-ray findings in one case *(14)*.

4. CYSTIC NEPHROMA

Cystic nephroma (CN) is an uncommon benign cystic neoplasm of adults and children. There have been numerous synonyms for this neoplasm including multilocular cyst and multilocular cystic nephroma. Originally thought to be a developmental abnormality, CN is now considered to be a neoplasm. A histologically similar neoplasm that also contains microscopic nephroblastoma is present in children and will not be considered further. CN occurs mostly in women over 30 yr old. The ratio of females to males for patients 5 yr of age and older is 8:1.

The tumor consists of a well-demarcated multilocular cystic mass separated from adjacent kidney by a fibrous capsule. CNs may herniate into the renal pelvis.

Microscopic criteria for the diagnosis of CN have changed somewhat over the years. Eble and Bonsib defined the most recent criteria as follows. CNs occur in adult patients and are expansile masses surrounded by a fibrous pseudocapsule. The interior is entirely composed of cysts and septa with no expansile solid nodules. Cysts are lined by flattened, hobnailed, or cuboidal epithelium. Septa may contain epithelial structures resembling mature renal tubules, but no epithelial cells with clear cytoplasm. Septa may not contain skeletal muscle fibers *(16)*.

By 1968, eight cases of sarcoma developing in CN were reported. At least three of the patients died of metastases. These sarcomas have been reported as undifferentiated embryonal spindle cell sarcoma (six cases), low-grade leiomyosarcoma (one case), and pleomorphic high-grade sarcoma *(16)*. Other than tumors in which sarcomas have developed, CNs have behaved in a benign fashion.

It is important to recognize this entity and to distinguish it from multilocular cystic renal cell carcinoma. In the latter tumor, neoplastic clear cells line some of the cysts.

5. RENAL SARCOMA

Primary non-Wilms' renal sarcoma (RS) accounts for approximately 1% of malignant tumors of the kidney and may involve the capsule, parenchyma, and renal pelvis. RS generally occurs in patients older than 60 yr of age. Sarcomas may also originate in perirenal fat. The three largest series of RS were reported from the Mayo Clinic, Memorial Sloan-Kettering Hospital, and MD Anderson Hospital *(17–19)*. The total number of RS in these series is 54, discounting five perirenal liposarcomas *(17)*. In the three series, the histologic distribution was as follows: leiomyosarcoma 31 (57%), hemangiopericytoma 7 (13%), unclassified 6 (11%), rhabdomyosarcoma 4 (7%), fibrosarcoma 3 (6%), and malignant fibrous histiocytoma 3 (6%). These studies were reported in 1976, 1984, and

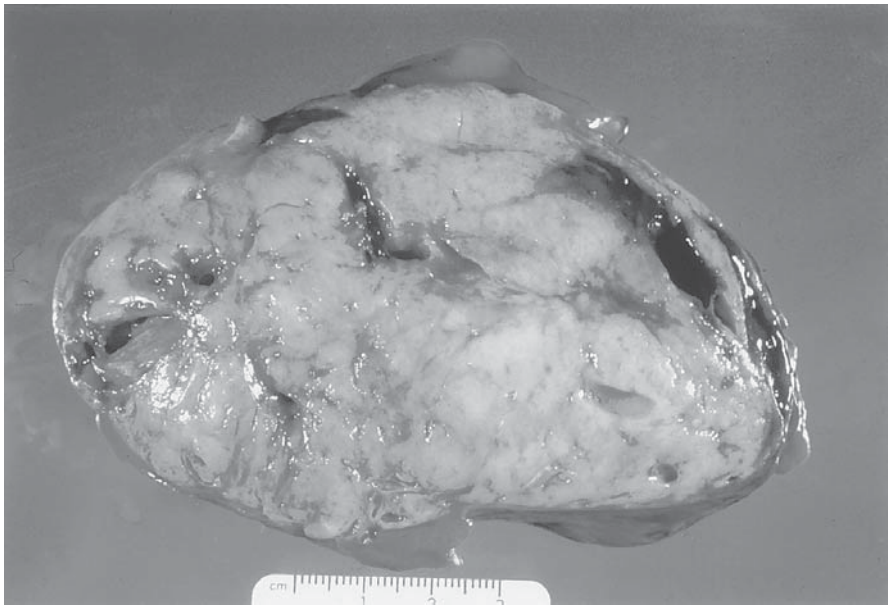

Fig. 15. Angiomyolipoma. 11-cm partial nephrectomy specimen. The mass is diffusely yellow-red with hemorrhagic foci.

1990. The classification of mesenchymal neoplasms has undergone considerable modification and refinement in the past decade and very probably the histologic diagnoses would change in some of these tumors if they were reviewed. Irrespective of the specific diagnosis, the prognosis of these tumors is poor. Only 4 of 54 patients in the above series were alive without disease, and one of those had only a 10-mo follow-up. Grignon et al. did DNA ploidy studies and found that aneuploidy correlated with histologic grade and nuclear pleomorphism, but not with survival *(19)*.

It is important to distinguish primary RS from angiomyolipoma (AML) and sarcomatoid renal cell carcinoma (RCC). AML has a far better prognosis and different therapy. Although sarcomatoid RCC also has a poor prognosis, therapy differs from that of primary RS.

6. ANGIOMYOLIPOMA

Angiomyolipoma (AML) is considered to be a hamartomatous mass, although Eble considered it to be a clonal neoplasm *(20)*. AMLs occur sporadically and in association with tuberous sclerosis (TS). Sporadic tumors are usually solitary and unilateral, whereas TS-associated tumors are generally multifocal and often bilateral. AMLs may be symptomatic or asymptomatic.

The gross appearance of AML reflects its histologic composition and depends on the relative amounts of blood vessels, smooth muscle or adipose tissue (Fig. 15). Tumors that are largely comprised of adipose tissue are yellow. Those that are largely comprised of muscle are gray-tan and resemble fibrous tumors. AMLs may be bright red as a result of massive hemorrhage and/or infarction. Some AMLs contain little or none of one element (e.g., angiomyomas are considered part of the AML family). AMLs occur in the capsule, cortex, or medulla and frequently extend into perirenal tissue. The tumors tend to have a sharp interface with renal tissue. AMLs are easy to recognize histologically if all the

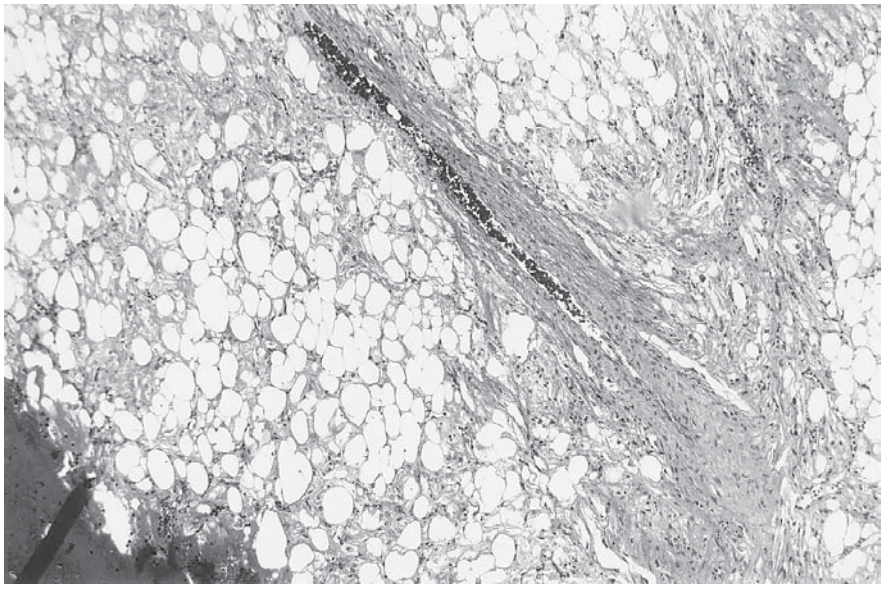

Fig. 16. Angiomyolipoma. Spindle cells develop from the wall of the blood vessel. Fat cells are extensive. H&E x65.

elements are present (Fig. 16). This is not always the case. Fat cells are generally mature, but may undergo fat necrosis. Blood vessels are usually thick-walled with less than the usual amount of collagen. Smooth muscle may grow in sheets or trabeculae and often appear to radiate from blood vessels. Smooth muscle is the most histologically variable element of AML and may demonstrate marked epithelioid atypia in benign tumors. Eble et al. and Martignoni et al. have described epithelioid variants in patients with and without TS *(21,22)*. This morphology may lead to an incorrect diagnosis of RCC, particularly in a biopsy. PAS-positive diastase-resistant crystalloid structures and granules have been described in AML *(23)*. Immunohistochemical stains are useful in the diagnosis of AML and in distinguishing them from RCC. AMLs usually stain positively for *HMB45*, a premelanosome marker, and for smooth muscle actin and muscle-specific actin *(24,25)*. Smooth muscle stains less frequently for desmin and negatively for keratin and epithelial membrane antigen. *HMB45* also stains smooth muscle cells of lymphangiomyoma, a tumor that may be present in TS, and tumor cells of malignant melanoma. Leiomyosarcomas stain negatively for *HMB45*.

AMLs may demonstrate aggressive behavior and may cause the death of the patient. The adverse effects of AML are massive retroperitoneal hemorrhage, invasion of contiguous organs, invasion of renal vein and vena cava, renal failure resulting from distortion or replacement of renal parenchyma, and metastasis. Despite these possible effects, few AMLs are truly malignant. Approx 40 cases of renal AML have associated AML in regional lymph nodes. These tumors have been considered benign and multifocal rather than metastatic *(26)*. A few cases have shown histologic evidence of sarcomatous transformation. Ferry et al. reported a case consistent with leiomyosarcomatous transformation and pulmonary metastasis *(27)*.

The differential diagnosis of AML includes RCC and leiomyosarcoma. When considering the diagnosis of AML, it is important to know whether the patient has TS. It is

important to recognize that patients with AML may also have RCC in the ipsilateral or contralateral kidney *(28)*. We have been RCC in approx 10% of our patients with AML.

7. ADULT WILMS' TUMOR

Adult Wilms' tumor (AWT), because of its rarity, may be confused with RCC. In 1980, Kilton et al. reported 192 cases, 35 of which contained pathologic data *(29)*. The diagnosis of AWT is easily made if the tumor is triphasic with blastemal, stromal, and epithelial components. Prognosis is poorer than that of Wilms' tumor (WT) in childhood. In Huser et al.'s review of 11 patients from 20 to 67 years of age, 45% presented with stage 3 and 4 disease, and 8 of 10 with adequate follow-up died of disease between 12 and 69 mo after surgery. The tumor was triphasic in seven and biphasic in four. No tumors had unfavorable histology by virtue of anaplasia *(30)*.

In adults, the differential diagnosis of AWT must include sarcomatoid variants of RCC, metanephric adenoma, neuroendocrine carcinoma, metastatic small cell carcinoma, and malignant lymphoma. Grossly, the tumor may be massive. AWTs tend to occur at higher stage than WTs in children. Microscopically, tumors are usually triphasic and biphasic. The diagnosis of a monophasic WT should be approached with skepticism. Metastases tend to occur. Most AWTs have favorable histology according to the definition of the National Wilms' Tumor Study.

8. UROTHELIAL NEOPLASMS OF THE RENAL PELVIS

Urothelial neoplasms of the renal pelvis (UN) also known as transitional cell neoplasms are relatively infrequent tumors accounting for about 8% of renal malignancies. These tumors are histologically similar to UNs of the bladder and should be classified according to modern concepts *(31)*. Tumors demonstrate the full range of neoplasia from urothelial papilloma including inverted papilloma through papillary urothelial carcinoma, urothelial carcinoma *in situ*, and invasive urothelial carcinoma (Figs. 17–19). Most UNs are associated with UNs elsewhere in the urinary tract. Invasive urothelial carcinomas are highly malignant and often rapidly fatal. Papillary urothelial tumors are usually borderline papillary tumors or low-grade urothelial carcinomas and are generally not lethal. They are often associated with and the patient's prognosis determined by urothelial tumors elsewhere in the urinary tract.

Urothelial carcinomas are usually easily distinguished from RCC and other renal neoplasms. Rarely, however, it is very difficult to distinguish an anaplastic carcinoma of urothelial origin from a sarcomatoid RCC. In these cases, it is most important to review the gross specimen to see if the neoplasm involves the collecting system. Microscopically, it is important to identify urothelial neoplasia elsewhere in the specimen. In an indeterminate neoplasm, multiple sections may be necessary to identify foci of UN or RCC. Rarely the distinction cannot be made even with multiple sections. Occasionally, an RCC may invade the renal pelvis and mimic a urothelial carcinoma. The distinction between urothelial carcinoma with glandular differentiation and collecting duct carcinoma may be difficult.

Additional tumors of the renal pelvis include adenocarcinoma, squamous cell carcinoma, combined small cell and urothelial carcinoma, osteoclastoma-like tumor associated with urothelial carcinoma, and hepatoid adenocarcinoma containing alpha fetoprotein *(32–34)*.

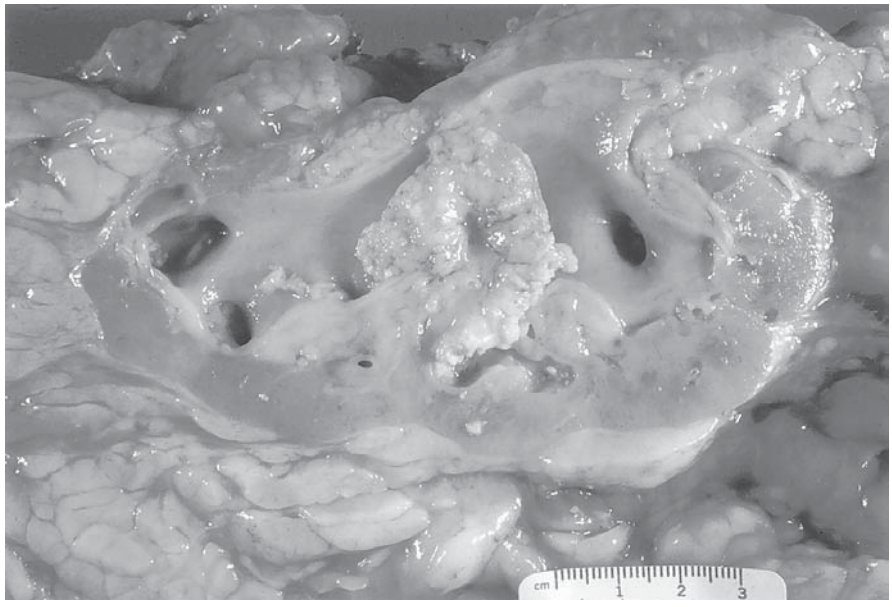

Fig. 17. Papillary and focally invasive low-grade urothelial carcinoma of the renal pelvis. A gray polypoid 3-cm mass projects into a dilated collecting system.

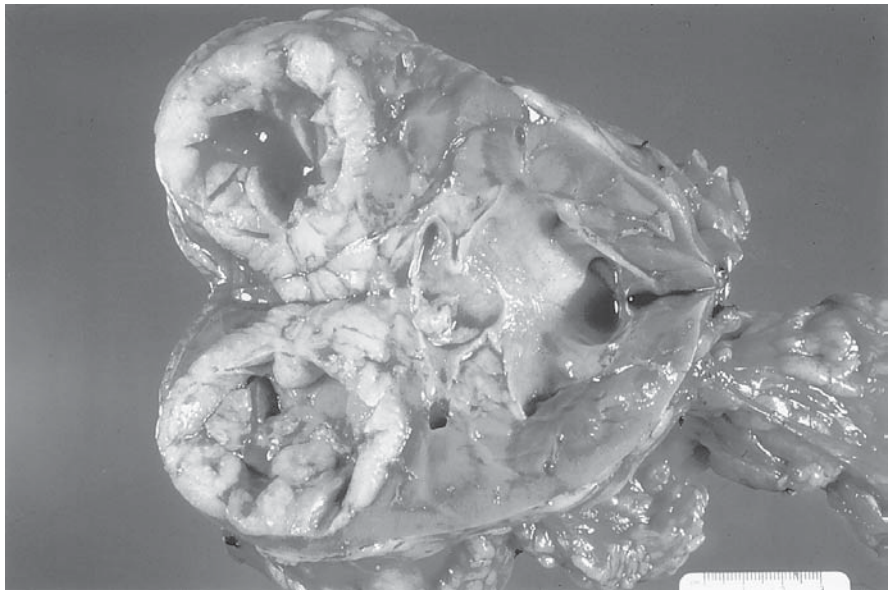

Fig. 18. Invasive high-grade urothelial carcinoma. A gray-white 6-cm firm neoplasm of the pole of the kidney infiltrates the renal parenchyma and renal sinus.

9. COLLECTING DUCT CARCINOMA

Collecting duct carcinoma (CDC), also known as Bellini duct carcinoma, is a primary renal neoplasm that differs from, and has a more malignant behavior than, RCC. Although these are rare neoplasms, more than 100 cases have been reported, and they are becoming

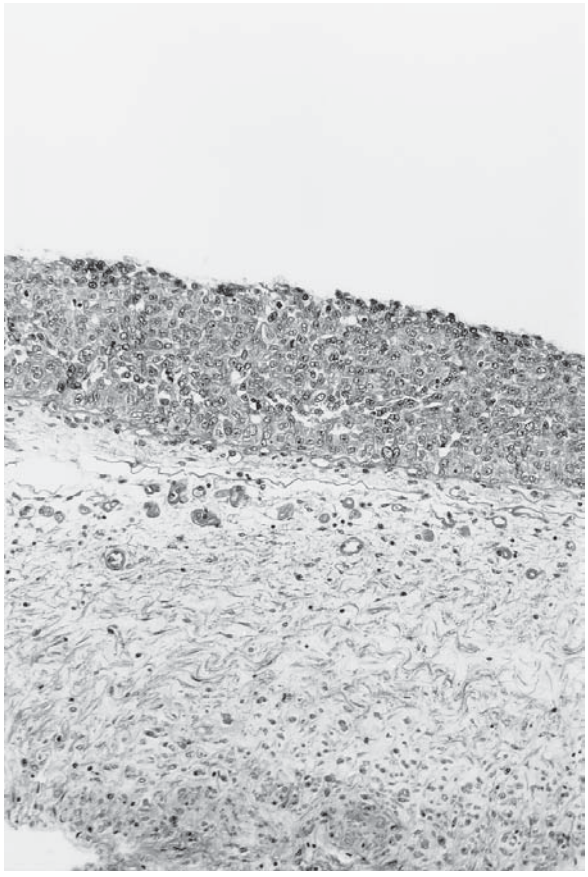

Fig. 19. Urothelial carcinoma in-situ of the renal pelvis. The urothelium of the renal pelvis shows full-thickness anaplasia. H&E x130.

increasingly recognized. CDCs have been reported in patients aged 13 to 83 yr with a mean age of 55 yr and a male-to-female predominance of approximately 2:1 *(35)*. Cases of similar histology in African-Americans may represent RMC.

CDCs are primarily located in the renal medulla and renal pelvis, but may invade the cortex (Fig. 20) *(36)*. They are usually gray-white, ill-defined, solid, and occasionally partly cystic with an infiltrating growth pattern. Microscopically, CDCs have a tubular, tubulopapillary, and papillary growth pattern with a desmoplastic stromal response, and often with numerous neutrophils (Fig. 21). Sarcomatoid CDCs have been described *(37)*. The individual cells contain enlarged irregular nuclei with prominent nucleoli and an increased nuclear-cytoplasmic ratio. These tumors closely resemble RMCs, but do not demonstrate sickled erythrocytes. Non-neoplastic collecting tubules nearby may demonstrate atypical hyperplasia or dysplastic epithelial cells.

Immunohistochemical stains may be diagnostically useful. Epithelial cells of CDC characteristically stain positively for high molecular-weight keratin (34 β E12), EMA, and Ulex Europeus agglutinin *(38)*. The tumor may be suspected on cytologic examination of urine or fine-needle aspiration when the cells do not resemble clear cell RCC. CDCs differ cytogenetically from RCC and have demonstrated monsomies of chromosomes 1, 6, 14, 15, and 22 *(39)*.

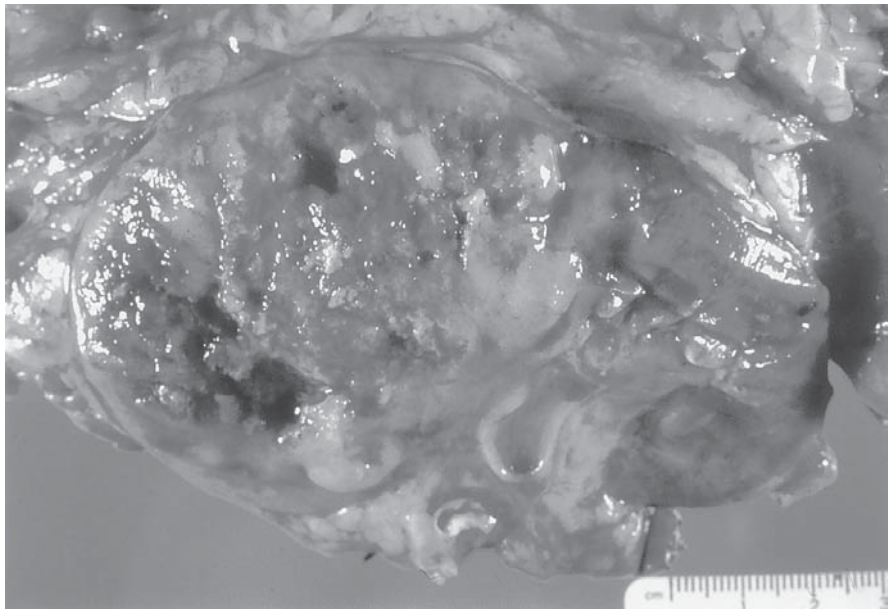

Fig. 20. Collecting duct carcinoma. A friable, necrotic, 7.5-cm, yellow-red-tan mass involves medulla, cortex, and pelvis.

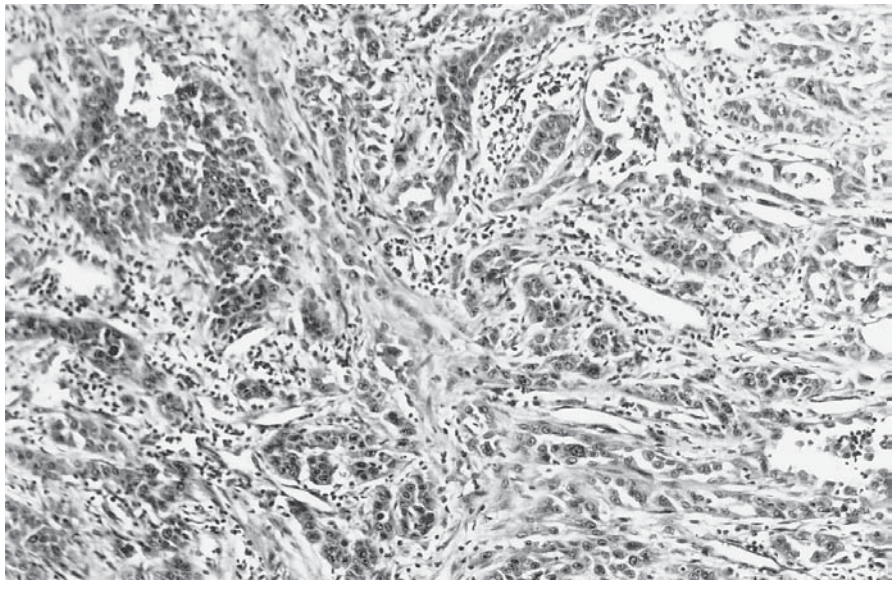

Fig. 21. Collecting duct carcinoma. Cords, poorly formed tubules, and solid aggregates of prominently nucleolated cells infiltrate an extensively inflamed stroma. H&E x130.

CDCs have an aggressive biologic behavior *(40)*. Lymph nodes with metastases may be present at the time of diagnosis. Matz et al. reported 10% of cases presented with lymph-node metastasis, especially to the cervical lymph nodes *(38)*. The prognosis of these tumors is poor with approx two-thirds of patients with collecting duct carcinoma dead of neoplasm within 2 yr following diagnosis. MacLennan et al. reported eight cases

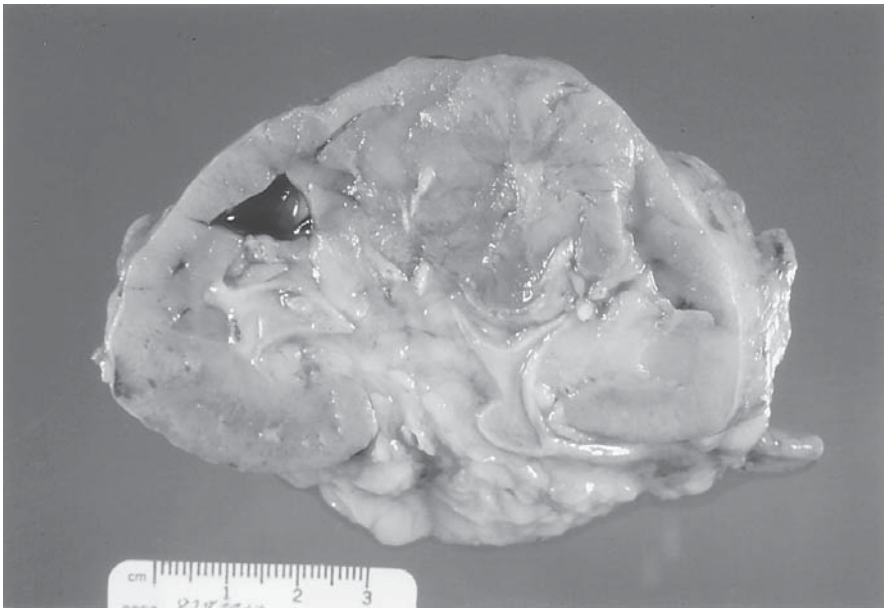

Fig. 22. Oncocytoma. The neoplasm is a golden brown 3-cm unencapsulated mass with a white central scar.

of low-grade mucin-producing tubulocystic renal carcinoma, which they suggested represented the lower end of the collecting duct carcinoma spectrum. Only one of these cases metastasized at 16 mo after surgery *(41)*.

10. ONCOCYTOMA

Although renal oncocytoma (RO) is a benign neoplasm, because of its resemblance to two varieties of RCC, it must be distinguished from RCC. ROs were originally characterized by Klein and Valensi in 1976 *(42)*. More than 300 cases have been reported in the medical literature and many more are unreported. ROs account for approximately 5% of renal neoplasms in adults. They are generally incidental findings, but may present with hematuria, mass, or pain.

The gross appearance of RO is of a discrete brown or mahogany-colored, circumscribed, but rarely encapsulated mass with a mean diameter of less than 6 cm and range from several mm to 24 cm (Fig. 22) *(43)*. ROs often contain a central scar. In addition, the neoplasm may contain fresh hemorrhage. The tumors may rarely extend into perirenal fat or veins. ROs may be multiple in up to 10% of cases. Rare cases of renal oncocytomatosis contain numerous oncocytomas that may occur bilaterally.

The microscopic appearance of RO is somewhat variable. The majority of neoplasms are comprised of small uniform cells with small nuclei and pink cytoplasm growing in an insular pattern within collagenous connective tissue (Fig. 23). The brown color of the gross specimen is reflected by the pink cytoplasm of tumor cells, which is rich in mitochondria. ROs may also grow in a microcystic, solid, or trabecular pattern. Atypical nuclei may be multiple, enlarged, and/or hyperchromatic. These findings are not associated with worsened biologic behavior (Fig. 24) *(43)*. Mitotic figures should not occur in RO. Occasional tumors may demonstrate gross and/or microscopic necrosis and fresh hemorrhage. ROs are almost invariably DNA diploid *(44)*.

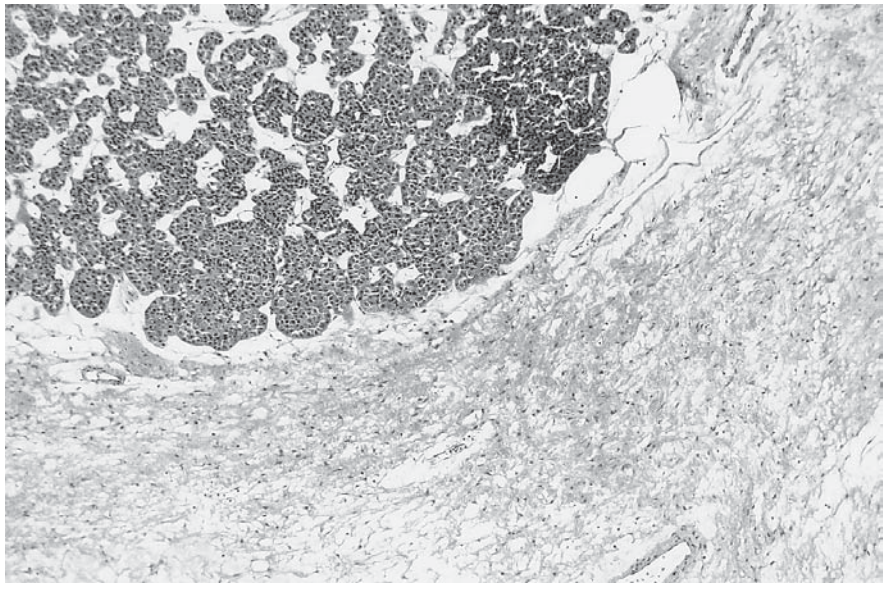

Fig. 23. Oncocytoma. The neoplasm is unencapsulated and composed of discrete insular groups of small uniform cells with pink cytoplasm. The tumor pushes into adipose tissue. This is not an adverse prognostic finding. H&E x65.

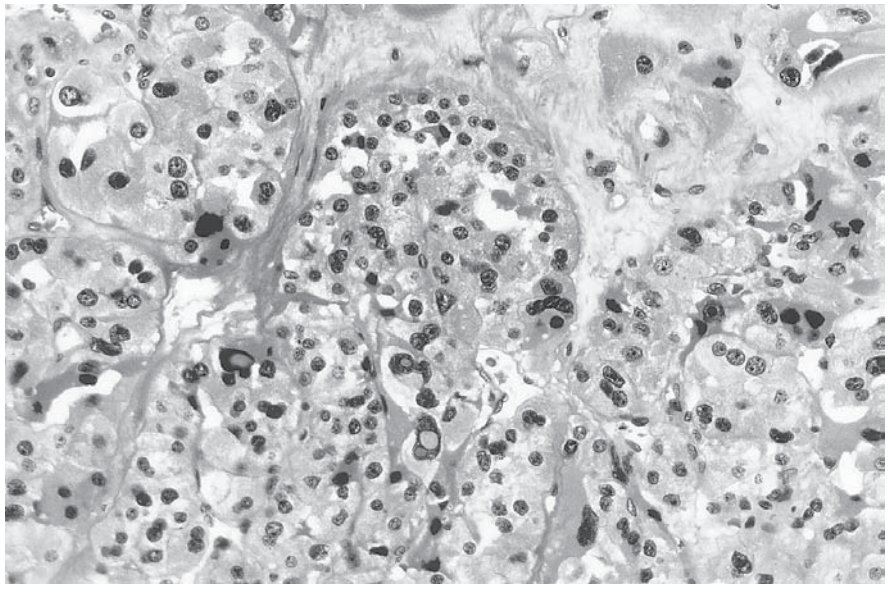

Fig. 24. Oncocytoma with nuclear atypia. Some nuclei are hyperchromatic and three times the diameter of most of the nuclei in an otherwise characteristic oncocytoma. This is not an adverse prognostic finding. H&E x260.

ROs have a biologically benign behavior and do not metastasize. It is important to distinguish ROs from chromophobe RCC and from the granular variant of clear cell RCC. In general, the distinction is not a problem, but occasionally the distinction is difficult. ROs sometimes coexist with other renal neoplasms, including papillary adenomas, angiomyolipomas, and RCC. The latter may be present adjacent to or within the

substance of an RO, or in the contralateral kidney. Fine-needle aspiration or needle biopsy is usually able to distinguish RO from RCC, but it is important to recognize that a given biopsy may not be totally representative of a kidney harboring both RCC and RO. In addition, tissue artifact or specimen inadequacy may preclude a definitive diagnosis.

11. METASTATIC CARCINOMA TO THE KIDNEY

Carcinomas from many sites may metastasize to the kidney. A 10-yr search of the English medical literature documented reports of clinically evident metastatic carcinoma from squamous-cell carcinoma, adenocarcinoma, and small-cell carcinoma of the lung, adenoid cystic carcinoma of salivary gland and external auditory canal, basaloid squamous-cell carcinoma of tongue, papillary and follicular carcinoma of the thyroid, adenocarcinoma of the colon, hepatocellular carcinoma, poorly differentiated carcinoma of the testis, gastric adenocarcinoma, adenoid cystic carcinoma of the breast, cervical carcinoma, adrenal cortical carcinoma, transitional cell carcinoma, and pheochromocytoma. Choriocarcinoma metastasizes relatively frequently to the kidney. Wang et al. reported 31 of 448 patients with choriocarcinoma had metastasis to the kidney *(45)*.

Symptomatology of metastatic carcinoma to the kidney includes bilateral renal enlargement and renal failure. Ultrasound or CT appearances favoring the diagnosis of metastatic carcinoma are multifocality, bilaterality, the presence of metastatic tumor elsewhere and coexistent perinephric tumor *(46)*. Metastasis to the kidney may also be silent and discovered at autopsy.

Even in the presence of metastatic carcinoma, the patient may be offered significant palliative relief or even cure depending on the nature of the tumor. As an example, choriocarcinoma may be cured with chemotherapy *(45)*. Fine-needle biopsy or core biopsy may be useful in making a specific diagnosis and in distinguishing metastatic carcinoma from a primary renal neoplasm. A specific diagnosis may enable specific therapy to be rendered or may spare the patient needless surgery.

12. RENAL LYMPHOMA

Although the kidney is commonly involved in disseminated non-Hodgkin's lymphoma (NHL), primary renal malignant NHL is rare. Hodgkin's disease virtually never occurs in the kidney. The large majority of NHLs involving the kidney are of B-cell type. Primary renal NHLs generally occur in middle-aged people, but may occur in children and adolescents *(47)*. The presentation of renal NHL may vary and includes abdominal or flank pain, a palpable mass, acute renal failure, or systemic symptoms.

Radiologic findings may vary and include a large retroperitoneal mass extending into the kidney, unilateral diffuse infiltration of renal parenchyma and rounded intraparenchymal masses *(48)*. Involvement may be unilateral or bilateral. Gross findings demonstrate fleshy or firm tan-gray masses that may invade contiguous structures (Fig. 25) *(47)*. Masses may be solitary or multiple. The microscopic findings consist of extensive interstitial infiltration by malignant lymphoma cells (Fig. 26).

The diagnosis of primary renal NHL must demonstrate the presence of a renal mass without initial extrarenal lymphomatous involvement, absence of a leukemic blood picture, and must have a histopathologic diagnosis *(49)*. Diagnosis of NHL should be made according to current internationally accepted classifications.

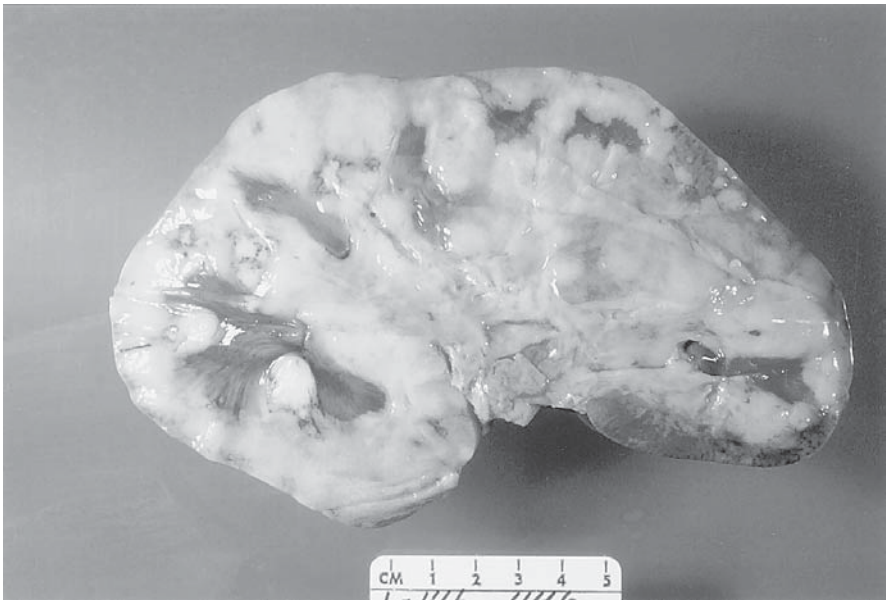

Fig. 25. Malignant lymphoma. Pale gray-white lymphomatous tissue diffusely infiltrates renal parenchyma.

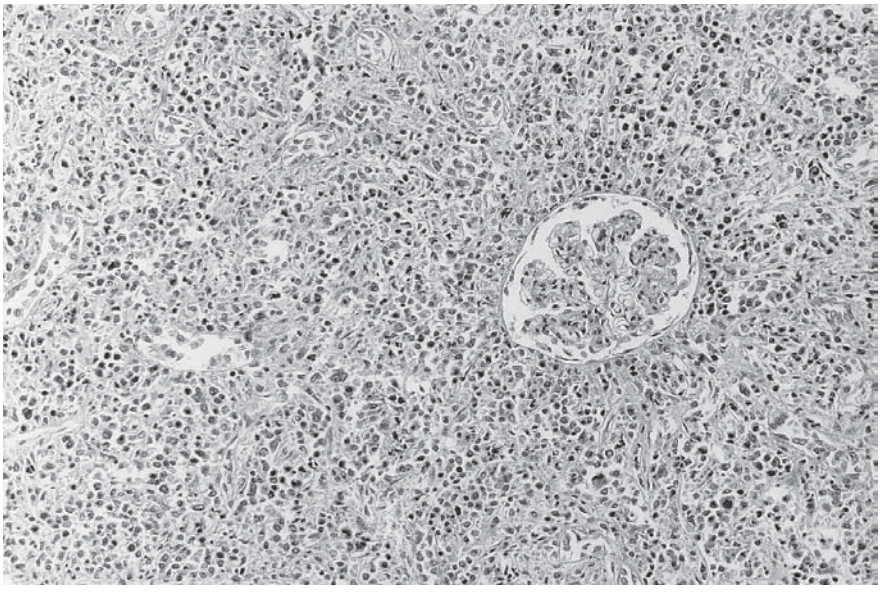

Fig. 26. Malignant lymphoma. There is massive extraglomerular interstitial infiltration by malignant lymphoma cells obliterating renal architecture. H&E x130.

NHL or postransplant lymphoproliferative disorders of the kidney may occur in patients with AIDS and in those with renal allografts *(50,51)*. Solitary plasmacytoma has also occurred in a renal allograft 14 yr after transplantation (52).

In cases where NHL involves the kidney, it is important to render a correct diagnosis. In different age groups, NHL may clinically mimic other tumors such as nephroblastoma

in children and RCC in adults. Bilaterality and typical radiologic findings may suggest the diagnosis of NHL, which can be confirmed by needle biopsy with tissue fixed in 10% formalin.

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3 Immunologic Response to Renal Cell Carcinoma

James H. Finke, Lisa Salvucci Kierstead, Elena Ranieri, and Walter J. Storkus

CONTENTS

1. CELLULAR IMMUNITY TO RCC

Based on more than 50 years of intensive research, we have come to the understanding that the ability of a host to reject an established tumor lesion depends on whether a cellular antitumor immune response can be effectively generated and maintained in that individual *(1–5)*. Murine tumor models readily demonstrated, that with few exceptions, the ability to confer protective antitumor immunity to naive mice is associated with the adoptive transfer of immune lymphocytes *(6–10)*. In marked contrast, the adoptive transfer of serum from tumor-immune animals into naive mice failed to confer resistance to tumor progression *(10,11)*. The critical nature of cellular antitumor immunity in the prevention and treatment of human malignancy has also been substantiated.

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In the clinical setting, several findings suggest that the immune system provides a safeguard against the development and progression of renal cell carcinoma (RCC) and may effectively mediate the regression of established lesions. Individuals undergoing systemic immunosuppression for the maintenance of transplanted organs exhibit increased incidence of RCC (largely derived from donor kidney allografts), suggesting the critical role of a functionally operational immune system in regulating tumor progression *(12–14)*. Furthermore, as is the case for melanoma, RCC lesions (such as those that undergo spontaneous regression) are typically infiltrated with large numbers of lymphocytes *(15–17)*.

Using semiquantitative reverse transcriptase polymerase chain reaction (RT-PCR) and primers designed to amplify cDNA encoding portions of the T-cell receptor (TCR) Vβ chain, clonally expanded populations of TCR α/β T cells have been readily demonstrated within tumor lesions *(18–20)*. Such expansions are believed to represent amplifications of RCC-reactive T cells *in situ* and are not observed in peripheral blood, normal kidney tissue, or tumor-draining lymph nodes *(18)*. Indeed, whereas clonal expansions of T cells can be demonstrated within primary and metastatic lesions within a single patient, these T-cell populations appear unrelated and likely exhibit altered specificities *(18)*. This may be the result of antigenic variation occurring in the context of the metastatic event, or the emergence of previously subdominant antigenic epitopes by the metastatic lesion. In addition to T cells expressing TCR α/β complexes, TCR γ/δ, and *NK/LAK* cells are also observed in RCC lesions *(21)*.

In immunotherapeutic approaches implementing tumor-based vaccines, delayed-type hypersensitivity (DTH) responses mediated principally by T lymphocytes have been observed at the site of subcutaneous or intradermal vaccine injection sites *(22–24)*. In addition, RCC-reactive cytolytic T cells have been identified in the peripheral blood and draining lymph nodes of vaccinated patients *(25,26)*. Last, the adoptive transfer of autologous T cells expanded ex vivo from resected tumor lesions or from tumor vaccine-draining lymph nodes has resulted in the objective clinical regression of residual disease in a subset of patients with RCC *(25–29)*.

Additional circumstantial evidence supporting the protective immunosurveillance against tumor progression may be discerned in the antigenic heterogeneity of phenotypes observed in RCC lesions *in situ*. Although addressed in far greater detail in an alternate chapter in this volume (Chapter 4: Immune Dysregulation), the finding of RCC foci containing cells that have reduced or lost expression of the TAP/LMP proteins or RCC-associated antigens required for effective recognition of RCC by CD8+ CTL strongly argues for active immunoselection in vivo during the tumorigenic process *(30)*.

2. LOCOREGIONAL IMMUNOCOMPETENCE AND TH1/TC1 VS TH2/TC2 ANTI-RCC T-CELL-MEDIATED IMMUNITY

Despite the frequently observed presence of leukocytes within RCC lesions, a clear correlate with beneficial clinical outcome in immunotherapeutic approaches has not been forthcoming *(31)*. In particular, clear cell and chromophilic RCC are typically heavily infiltrated with T (CD4+ and CD8+) lymphocytes *(32)*, however, the composition of these infiltrates suggest that they may frequently be affiliated with a suboptimal anti-tumor immune response that is unable to slow tumor progression or lead to tumor regression. This finding may be partially related to locoregional tumor immunosuppression

(detailed in Chapter 4: Immune Dysregulation) and partially to the nature of the immune response evolving within the tumor and the tumor-draining lymph node(s).

Mature CD4+ T-helper cells can be segregated into two principal cytokine categories, Th1 and Th2, based on cytokine secretion *(33)*. More recently, cytotoxic CD8+ T cells have been similarly discriminated into Tc1 and Tc2 categories, based on their abilities to preferentially secrete IFN-γ (Th1,Tc1) or IL-4/IL-5 (Th2,Tc2) *(33,34)*. Although this dichotomy in T-cell subsets was originally documented in the mouse, recent reports support a similar segregation of such T-cell subpopulations in humans *(34)*. In general, the ability of an organism to mount a Th1-biased immune response has been affiliated with enhanced ability to eliminate certain pathogens and tumors *(35,36)*. IL-12 (produced by dendritic cells, *[37]*), in particular, appears to play an important role in determining whether a Th1- or Th2-biased immune response is mounted to antigenic challenge. Functionally, IL-12 has been demonstrated to bias the immune response toward a Th1 phenotype in vitro and in vivo *(38)*. In addition to promoting Th1 development from naive and memory T-cell populations, IL-12 has also been shown to suppress the development of Th2 cells *(39,40)*. Interestingly, RCC secrete IL-6 *(41)*, and high systemic levels of IL-6 have been correlated with poor prognosis and survival of patients with RCC *(42)*. This may in part be caused by the ability of IL-6 to suppress production of IL-12 *(43)* and subsequent Th1-type immune production of IFN-γ. Indeed, TIL harvested from RCC have been demonstrated to be hyporesponsive to exogenous IL-12 *(44)*.

The data addressing the issue of Th1/Tc1 vs Th2/Tc2-biased immunity occuring in RCC patients are equivocal *(20,45–50)*. In some studies, freshly isolated RCC TIL may exhibit a predominant Th2/Tc2-type phenotype associated with the locoregional production of IL-4 and IL-10 *(45–50)*. These cytokines are affiliated with enhanced humoral (i.e., antibody) responses and with inhibition of "professional" antigen-presenting cell (APC) (i.e., dendritic cell [DC]) function, respectively, and are often inversely correlated with effective induction or dysfunction of cellular T-cell-mediated immunity. However, Angevin et al. *(20)* have shown in 12 patients with primary RCC that isolated TIL are strongly polarized to a Th1/Tc1 differentiation pattern (i.e., production of IL-2 and IFN-γ), but may be suppressed in vivo by strong production of IL-6/IL-10 within the tumor microenvironment *(20,45,47,49)*. These data support a mixed Th1/Th2-type T-cell infiltrate that may overall display Th2-type T-cell function. This provides confidence that immunotherapeutic strategies capable of diminishing Th2/Tc2- and enhancing Th1/Tc1-type T-cell function within the RCC lesion may provide significant clinical benefit.

3. INDUCTION OF ANTI-RCC T-CELL IMMUNITY

The ability to initiate the cellular immune response is brokered by sentinel APC found at the site of antigen exposure (i.e., in the tumor microenvironment). The most dominant "professional" APC, the DC, conveys antigens from the periphery to draining lymph nodes where they may efficiently prime and expand antitumor T cells. Based on a large number of histopathology surveys of various cancer histologies, the number of DC found within tumor lesions is often inversely correlated with the disease grade and directly correlated with patient survival *(51–55)*. Troy et al. *(56)* have evaluated the functional immunophenotype of DC isolated from RCC in eight patients. Most "mature" CD83+ DC were located at the periphery of the tumor in association with T-cell clusters, whereas few

CD83+ DC were located within the tumor. However, Thurnher et al. *(57)* found that substantial numbers of DC emigrate from RCC explants in organ cultures, with up to 9% (i.e., 40-fold-higher frequency than peripheral blood) of emigrating leukocytes exhibiting a CD83+, CD86+, CD45RO+ "mature" DC phenotype. Although these cells were poor at antigen acquisition, they were competent to prime naive T cells in vitro. Cumulatively, these data may support the presence of significant numbers of functionally suboptimal DC within advanced RCC lesions, that may result in the suboptimal induction (within tumor draining lymph nodes) and maintenance (within tumors) of T-cell-mediated anti-RCC immunity.

4. CHARACTERIZATION OF THE CELLULAR IMMUNE RESPONSE TO RCC

The mechanism by which lymphocytes, and in particular, tumor-reactive T cells expressing TCRα/β complexes recognize antigen(s) is now well described *(58–60)*. The vast majority of antitumor T cells recognize tumor antigens as short protein fragments or peptides presented on the tumor cell surface by major histocompatibility complex (MHC) class I (present 8–12 amino acid long peptides) and class II (present somewhat longer peptides up to approx 35 amino acids in length) molecules *(59,60–62)*. These peptides may derive from virtually any proteins synthesized by the tumor cell (i.e., proteins that are found in the nucleus, cytoplasm, lysosome, plasma membrane, or that are secreted), only a small number of which might represent "tumor-associated" or "tumor-specific" sequences. Although still located within intracellular compartments, these tumor peptides associate with nascent MHC class I or class II molecules and are subsequently transported to the cell surface where they become accessible to CD8+ and CD4+ T-cell scrutiny, respectively *(63,64)*. The ability of a given peptide to bind to, and be presented by, a given MHC allele is determined by structural motifs within the peptide sequence (defining a "peptide binding motif") that allow for sufficient compatibility between peptide amino acid side-chains and micropockets formed within the peptide-binding groove of the MHC molecule *(65–68)*. This degree of intermolecular compatibility determines the affinity of peptide for an individual MHC molecule, the corresponding half-life of such stable complexes and, to a large degree, the likelihood that the peptide-MHC complex is immunogenic to the existent T-cell repertoire *(69–71)*. Overall, only a limited number (i.e., 1–200) of specific peptide-MHC complexes need to be expressed by a tumor cell target to allow for T-cell effector function to be induced *(72–74)*.

Unlike melanoma, where it has generally proven straightforward to generate tumor-specific T-cell lines and clones either from TIL or from the peripheral blood of patients, RCC has typically been far more refractory to such attempts, with nonspecific effector cells the result of most MLTC (mixed lymphocyte plus tumor cell) cultures. One method that has enhanced the generation of MHC-restricted CTL has been the in vitro application of RCC stimulator cells that have been genetically engineered to express T-cell stimulatory cytokines (such as IL-2 or IFN-α) or T-cell costimulatory molecules (B7-1, CD80) in MLTC protocols *(75–79)*. A summary of published data describing RCC-reactive CTL lines and clones is provided in Table 1. Overall, these results suggest that HLA class I-restricted CD8+ T cells recognizing RCC typically react against "shared" antigenic epitopes since both autologous and HLA-matched RCC cell lines are lysed *(80–85)*. In

Table 1
Characterization of Anti-RCC CD8+ T-Cell Reactivities

				Targets Evaluated							
CTL	*Line/ Clone*	*Tissue Origin*	*HLA Restriction*	*RCC*	*Auto RCC*	*Allo NK*	*Auto EBV*	*Auto PHA-T*	*K562*	*Other*	*Ref.*
ND	Line	Tu	NE	+	-	NT	-	NT	-	NT	*(80)*
ND	Line	Tu	A2	+	+	-	NT	NT	-	NT	*(81)*
CTL5	Line	PBL	A2	+	+	+	-	-	-	A	*(82)*
5-10	Clone	PBL	B8	+	+	+	-	-	-	-	*(83)*
5-30	Clone	PBL	A2	+	+	+	-	-	-	A	*(83)*
VIIB10	Clone	Tu	NE	+	NT	+	NT	-	NT	NT	*(19)*
VIIC2	Clone	Tu	NE	+	NT	-	NT	NT	NT	NT	*(19)*
5E	Clone	Tu	B37	+	+	-	NT	NT	-	NT	*(84)*
263/17	Clone	PBL	B7	+	+	-	NT	NT	-	NT	*(84)*
263/45	Clone	PBL	B7	+	+	-	NT	NT	-	NT	*(84)*
6/135	Clone	PBL	A1	+	+	+	-	NT	-	B	*(85)*

ND = Not Designated; NE = Not Established; NK = Normal kidney; proximal tubule epithelium; NT = Not Tested; PBL = Peripheral blood lymphocytes; Tu = Tumor; A = Melanoma; B = Melanoma, mesangial cells, breast epithelium.

approximately half of the cases, CD8+ CTL recognized both autologous RCC and cultured normal kidney epithelium, supporting a target antigen that may represent a renal differentiation antigen or a broadly expressed "self" antigen. This latter scenario may be supported in the cases of CTL clones 5-30 and 6/135, that recognize both melanoma and RCC tumor cell lines. Whereas the target cell range of reactivity is limited for the CTL line described by Finke et al. *(80)*, this T-cell population appears to recognize an idiotypic RCC epitope because only autologous RCC was recognized in the cytolytic analysis performed.

5. IDENTIFICATION OF RCC-ASSOCIATED CD8+ T-CELL-DEFINED ANTIGENS AND EPITOPES

Our ability to molecularly define the RCC-associated antigens capable of being recognized by the cellular immune response has advanced considerably in the past decade. Whereas the vast majority of emphasis has been directed toward the identification of CD8+ CTL-recognized antigens/epitopes, more recent attempts have focused on the characterization of the targets of CD4+ "helper" T cells (in particular Th1-type CD4+ T cells) *(86)*. Three principal schemes have been used successfully to isolate and characterize the genes encoding tumor antigens and their derivative peptide epitopes: (1) expression cloning of tumor-associated cDNAs using patient PBL- or TIL-derived antitumor T-cell lines; (2) mass spectrometric "sieving" of tumor MHC-presented peptides recognized by T cells; and (3) expression cloning of tumor-associated cDNAs using patient-derived antitumor IgG antibodies (i.e., an Ig isotype that requires CD4+ T-cell "help"). This latter approach has been termed "SEREX" denoting the serologic expression cloning technique *(87–126)*.

The first, and currently most successful approach, was pioneered by the laboratories of Thierry Boon and Pierre Van der Bruggen (Ludwig Institute, Brussels, Belgium) and Steve Rosenberg (NCI, Bethesda, MD) and involves the transfection of tumor-derived cDNA into a recipient cell line that is cotransfected with a cDNA encoding a relevant MHC class I allele. The resulting transfected cell is capable of presenting tumor antigen-derived peptides to MHC class I-restricted CTL. The ability of the transfectants to elicit the secretion of cytokine (typically tumor necrosis factor (TNF)-α) from tumor-specific T cells allows for the identification of the tumor-associated antigen (TAA) cDNA, which may then be sequenced. Based on the cloned sequence of the melanoma-associated protein and the putative peptide-binding motifs identified for specific MHC alleles, a series of synthetic peptides may be generated to determine the actual peptide epitope recognized by the original CTL line or clone used in the screening process (Table 2). Cumulatively, these antigens have fallen into four main categories: (1) cancer-testis (CT) antigens that are expressed by tumor cells and only by testis and placenta among normal tissues; (2) lineage-restricted antigens that are expressed by normal tissues, but that may be overexpressed by tumor cells; (3) antigens that are expressed or overexpressed by tumors cells of mutiple lineages; and (4) antigens that are mutated in tumor cells.

In the second approach, peptides are extracted from tumor cell MHC complexes by acid treatment and then analyzed by mass spectroscopy, a sensitive peptide sequencing method capable of evaluating femtomolar concentrations of individual peptide species within complex mixtures of proteins. By sequencing, synthesizing, and screening a large number of such peptides for their ability to reconstitute T-cell recognition when pulsed onto

lymphoid target cell lines (MHC class I matched with responder CTL), this approach has been successfully used to identify naturally-processed T-cell epitopes derived from RAGE-1 in RCC *(121)* and MART-1, gp100, tyrosinase in melanoma *(94,109,125)*.

In the third approach, serum antibodies isolated from the blood of patients with cancer is used as an indicator reagent in cDNA expression cloning (SEREX). In particular, the use of serum IgG antibodies that require the generation of CD4+ T "helper" T cell responses to promote isotype switching in Ig-secreting B blasts has been implemented. Although this approach was initially used to successfully identify the NY-ESO-1 melanoma antigen that is a member of the *CT* gene family and encodes CD8+ T-cell recognized epitopes (*114–116*, Table 2), this approach has generic applicability and may be readily applied to RCC. No RCC-specific antigens have been thus far identified using this technique. However, based on the theory of SEREX, RCC-associated antigens that have been traditionally used as serologically defined histopathology markers, such as the G250 antigen *(127)*, should represent candidate RCC antigens that yield T-cell-defined epitopes.

The vast majority of tumor-associated antigens that have been identified to date are expressed by melanoma cells because they served as the prototype model for the initial application of the underlying "classical" screening protocols outlined above. Based on the finding of RCC-derived CTL crossreacting with melanoma (Table 1), it is likely that some antigens initially identified as melanoma-associated antigens will also prove to be RCC-associated antigens (i.e., multilineage tumor antigens). In addition, a number of antigens expressed preferentially or exclusively by RCC or by many tumor histologies (including RCC) have been identified in the past five years. The first "RCC-associated antigen" defined as a T-cell target was the *RAGE-1* gene product. *RAGE-1* is expressed by approximately 2–20% of primary RCC and in a small percent of sarcoma, bladder carcinoma, and melanoma *(90,120)*. Peptides derived from one ORF (*ORF2*) have been shown to be presented in the context of *HLA*-B7 to CD8+ CTL *(120)*, whereas a second *RAGE-1* epitope derived from *ORF5* and presented by *HLA*-B8 has recently been identified *(121)*. Other RCC-expressed antigens yielding peptide epitopes recognized by T cells include *Her-2/neu (97–99)*, *PRAME (90,118,119)*, *gp75 (90)*, *MUC-1 (108)*, a mutated *HSP-70* molecule *(100)*, and a protein generated by an alternate reading frame of the intestinal carboxyl esterase gene mRNA *(88)* (Table 2).

Despite this recent success in identifying RCC-associated antigens using approaches outlined above, the inability to generate long-term RCC-specific T-cell lines for use as "read-out" reagents may prohibit the rapid prospective identification of additional RCC-associated antigens. As a possible alternative strategy, single-cell T-cell screening systems, such as the cytokine ELISPOT, allow for the identification of tumor-associated peptides using "memory" CD4+ and CD8+ T cells isolated directly from the patient's blood *(128–130)*. By analyzing the freshly isolated immune response to tumor-derived antigens, one is not prone to any potential artifact associated with extended in vitro culture of effector T cells that may deviate the repertoire of the bulk antitumor T-cell response. A careful selection of cytokines such as IFN-γ and IL-5 for ELISPOT analysis, allows for the rapid diagnosis of the Th1/Tc1 vs Th2/Tc2 bias, respectively, of the patient's basal immune response to autologous tumor. Furthermore, by performing such scans of patient PBL-derived T-cell responses during immunotherapy and subsequent follow-up, one may effectively monitor and correlate tumor-specific immunity with clinical disease course in a manner that is reflective of the repertoire of anti-RCC reactive T cells *in situ*.

Table 2
Tumor-Associated Antigen (TAA) Epitopes Defined By HLA-Restricted T Cells

Antiten Defined	*TAA Family*	*Epitope Sequence*	*HLA Restriction Allele*	*% Melanoma Expressing*	*% RCC Expressing*	*Subcellular Location*	*Obj. CR*	*Ref.*
BAGE	CT	AARAVFLAL	Cw1601	22%	0%	CYT?		*(87)*
Carboxyl esterase*	LR	SPRWWPTCL	B7	0%	75%	ND		*(88)*
b-Catenin	MUT	SYLDSGIHF	A24	mutant	0%	PM		*(89,90)*
CDK4-kinase	MUT	ACDPHSGHFV	A2	mutant	NT	N		*(91)*
GAGE-1/2	CT	YRPRPRRY	Cw6	24%	0%	CYT?		*(92)*
gp75 (TRP-1)	LR	MSLQRQFLR	A31	46%	11%	MLS	Y	*(90,93)*
gp100	LR	YLEPGPVTA	A2	54%	0%	MLS	Y	*(90,94–96)*
		LLDGTATLRL	A2					
		KTWGQYWQV	A2					
		ITDQVPFSV	A2					
		VLYRYGSFSV	A2					
Her2/neu	ML	KIFGSLAFL	A2	58–75%	40-45%	PM		*(97–99)*
		IISAVVGIL	A2					
HSP70-2	MUT	SLFEGIDIYT	A2	NT	mutant	CYT		*(100)*
MAGE-1	CT	EADPTGHSY	A1	36–50%	0-22%	CYT	Y	*(70,90,101–103)*
		SAYGEPRKL	Cw1601					
MAGE-3	CT	EVDPIGHLY	A1	64–79%	76%	CYT	Y	*(90,104–106)*
		FLWGPRALV	A2					
		MEVDPIGHLY	B44					
MART-1	LR	AAGIGILTV	A2	90%	0%	MLS		*(90,107–110)*
		ILTVILGVL	A2					

MUC1	ML	LLLLTVLTV	A2	0%	30-40%	PM		*(111)*
		STAPPVHNV	A2					
MUM-1	MUT	EEKLIVVLF	B44	mutant	0%	ND		*(90,112)*
NAG-V	ML	VLPDVFIRC	A2	50%	NT	GOL		*(113)*
NY-ESO-1	CT	SLLMWITQCFL	A2	34%	0%	CYT?		*(90,114–116)*
		SLLMWITQC	A2					
		QLSLLMWIT	A2					
p15	ML	AYGLDFYIL	A24	NT	NT	ND	Y	*(117)*
PRAME	CT	LYVDSLFFL	A24	93%	41%	CYT?		*(90,118,119)*
RAGE-1 (ORF2)	CT	SPSSNRIRNT	B7	0%	2-21%	CYT?		(*90,120,121)*
(ORF5)		PASKKTDPQK	B8					
Tyrosinase	LR	MLLAVLYCL	A2	80–94%	0%	MLS	Y	*(90,121–126)*
		YMDGTMSQV	A2					
		YMNGTMSQV	A2					
		AFLPWHRLF	A24					
		SEIWRDIDF	B44					

CT = Cancer-Testis antigen, CYT = cytoplasm, GOL = golgi, LR = Lineage-Restricted antigen, ML = Multi-Lineage antigen, MUT = Mutated antigen, N = nucleus, PM = plasma membrane, MLS = melanosome/lysosome, ND = not determined. Clinical trials in which complete regressions have been demonstrated upon adoptive transfer of T cells reactive with TAA or by vaccination with peptide epitopes derived from TAA is designated by (Y). Mutant peptide epitopes are unique to a given patient melanoma and are not generally shared by other unrelated melanoma. *Intestinal carboxyl esterase epitope generated from alternate reading frame by translational "slippage" (Ronsin+)

6. IDENTIFICATION OF RCC-ASSOCIATED CD4+ T-CELL-DEFINED ANTIGENS AND EPITOPES

CD4+ T cells clearly play a critical role in the complex biologic equation yielding antitumor immunity *(86)*. A positive correlation has been noted between the intensity of lymphocyte infiltration and the percentage of CD4+ T cells in RCC TIL *(21)*. In addition, the CD4+/CD8+ T-cell ratio in the peripheral blood of patients that exhibit objective clinical responses (OCR) to IFN-α therapy is drastically increased concomitantly with regression of RCC lesions and is associated with enhanced patient survival *(131)*. Helper CD4+ T cells in RCC TIL have an activated, memory phenotype *(132)*, consistent with their potential effector role within the tumor microenvironment. In addition, certain HLA class II alleles (HLA-DR*0101 and -DR*0405) have recently been identified that may be associated with resistance to RCC development and progression *(133)*. This finding may suggest that these class II alleles are particularly adept at presenting RCC-derived peptides to Th1-type (or less likely, particularly poor at stimulating Th2-type) CD4+ effector cells that efficiently promote and maintain anti-RCC immunity in certain HLA-DR1+ or -DR4+ patients.

Despite this clear importance in the underlying generation and maintenance of anti-RCC immunity, no RCC-associated peptides that serve as CD4+ T-cell epitopes have been described to date. Given the recent interest in characterizing "helper" T-cell epitopes in alternate tumor histologies *(86,134–136)*, this will clearly represent an active area of prospective study. In particular, it will be critical to determine the nature of Th1- vs Th2-type CD4+ T-cell recognition of RCC-derived epitopes and delineate how we may immunotherapeutically enhance a Th1-type bias in patient responses

7. ACTIVE IMMUNOTHERAPY OF RCC: VACCINES

RCC represents a tumor histology that has proven highly resistant to traditional chemotherapeutic and radiotherapeutic regimens *(27,137,138)*, which has necessitated the development of alternate clinical strategies. Although objective clinical response rates associated with immunotherapeutic approaches (vaccines, systemic application of biologic response modifiers, such as IL-2 and IFN-α) have typically fallen in the 10–20% range *(22–29,139,140)*, it appears clear that these protocols have the greatest degree of promise in providing durable responses in patients with advanced disease *(140)*. The overall success of immunotherapies will also greatly benefit from the increased incidental diagnosis of RCC *(141)*, with up to 30% of RCC identified early in tumor evolution. This allows for the application of immunotherapies in the context of smaller tumor burdens in patients presenting with a comparably high-degree of immunocompetence (vs patients with advanced disease). Furthermore, prophylactic vaccines may be envisioned for individuals at high risk to develop RCC, such as those who exhibit a familial inherited form of RCC (rare autosomal dominant), individuals with Von-Hippel Lindau (VHL) syndrome, and potentially those patients with tuberous sclerosis or autosomal dominant polycystic kidney disease *(142–144)*.

Several vaccination protocols have been used previously in the adjuvant setting of RCC, such as vaccines consisting of inactivated autologous tumor cells admixed with BCG or with C. parvum, or viable autologous RCC fused with a cultured cell line *(23,24, 140–142)*. Interestingly, induction of positive immune response to vaccination was confirmed in enhancement of DTH responses to autologous tumor, but not normal kidney

tissue, injected subcutaneously *(23)*. Whereas whole (autologous or allogeneic) tumor cells were initially implemented as "antigen" in early vaccines, it is now feasible to apply molecularly characterized RCC-associated genes, proteins, and peptides (Table 2) as immunogens in such approaches. RCC-derived peptides have a significant technical advantage in that they are inexpensive to produce, biochemically well-defined and simple to apply. However, they suffer the significant disadvantage that a given RCC-derived peptide sequence is typically only presented by a single MHC allele, which may only be applied to that subset of patients that express that HLA allele (always less than 50%, i.e., HLA-A2 is the highest frequency allele expressed by approximately 45% of patients, *[145]*). The patient's tumor cells should also express the tumor antigen from which the vaccine-incorporated peptide derives, which may further diminish the generic utility of a single peptide-based vaccine strategy. Furthermore, it has been hypothesized that single epitope vaccines may prove therapeutically ineffective because epitope- and/or antigen-loss tumor variants may have been immunoselected in vivo from an antigenically heterogenous population of tumor cells during the typically long periods of time associated with tumorigenesis or during active immunization *(146–148)*. Although initial tumor regression might be observed in patients harboring such tumors, antigen- or epitope-loss variants might ultimately progress. However, it is also possible that single epitope-based vaccines may result in "epitope spreading" (see below) that may minimize such concerns.

In either case, it would be anticipated that vaccines predicated on synthetic CD8+ T cell RCC-derived epitopes would benefit from the inclusion of MHC-presented "helper" epitopes recognized by CD4+ T cells, in order to optimally activate or maintain the resulting CTL response *(86,149,150)*. Such epitopes may direct the generation of CD4+ cytolytic T cells or a DTH-like immune response mediated by tumor-specific CD4+ T cells within the tumor lesion that have been triggered as a result of MHC class II+ tumor cells or by tumor-localized MHC class II+ APC (i.e., dendritic cells) presenting RCC-derived epitopes (Fig. 1).

8. DC-BASED VACCINES: CROSS-PRESENTATION AND "EPITOPE SPREADING"

Human DC have recently been used successfully in a series of recent clinical protocols, principally for the treatment of melanoma, lymphoma, and prostate cancer *(151–153)*. These cells represent the prototypical APC type in the body and are uniquely characterized by their ability to capture antigens within their tissue microenvironment, to process and present antigen-derived epitopes in MHC class I and II complexes on their cell surfaces, and to migrate to draining lymph nodes where these APC may activate naive and memory antigen-specific T cells. Based on insights gained in a series of murine tumor models, a kinetic model of effective tumor vaccination may be hypothesized (Fig. 1). In this model, DC that have acquired injected, vaccine-associated tumor antigen, or DC that have been preloaded ex vivo with tumor antigen (i.e., peptide) and injected as a vaccine, migrate to vaccine site draining lymph nodes and promote the activation of tumor antigen-specific T cells. In the presence of DC-secreted cytokines, such as IL-12, a Th1-type immune response is augmented, resulting in enhanced antigen-specific CTL generation. These mature CTL may then leave the lymph node, recirculate, and recruit to tumor sites. Within the tumor microenvironment, these CTL may mediate the cytolysis or apoptosis of tumor cells that provides a new format of tumor antigen (i.e., tumor "lysate" or apoptotic tumor bodies) that may be acquired by tumor-infiltrating DC

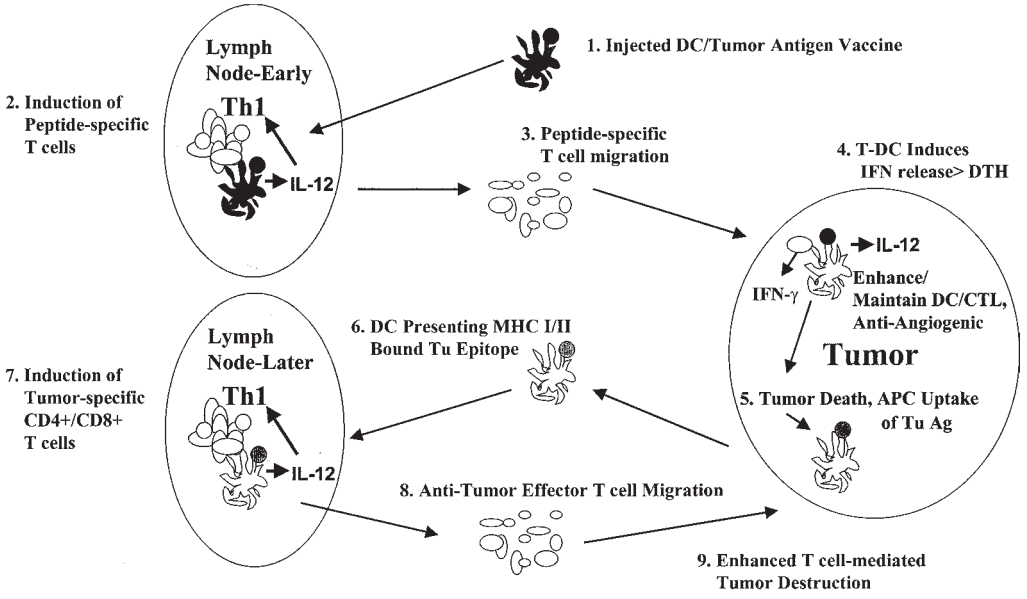

Fig. 1. DC-based vaccine-induced "epitope spreading." Based on the rationale provided in text, the initial vaccinated DC promote a primary wave of effector T cells that promote tumor necrosis and apoptosis. The tumor debris is engulfed by tumor-infiltrating DC, processed and presented as MHC-bound peptides, and transported to the draining lymph nodes where secondary antigen-specific T-cell induction/expansion may occur. These latter effector cells conceptually exhibit a far broader repertoire of tumor-relevant specificities and may serve to preclude immune escape by antigen-loss variants within antigenically heterogeneous RCC lesions.

(151,154,155). These newly accessed DC can then migrate to the draining lymph nodes and activate a secondary "wave" of CD4+ and CD8+ T-cell activation, resulting in a broadening of the repertoire of antitumor effector T cells (i.e., only a small percentage will recognize the original vaccine-associated peptide). This phenomenon, termed "epitope spreading" may preclude the generation of "antigen-loss" tumor variants, because a broad range of tumor antigenic epitopes will be targeted by the broadened immune response. This effect has been clearly documented in murine tumor models and in human studies *(156–158)*, and is likely to represent a major mechanism associated with tumor regression in human clinical responders. As such, clinical protocols using vaccines designed with a limited series of peptide epitopes or recombinant tumor antigen proteins, should include monitoring that allows for the detection of immunity directed against tumor-relevant, but vaccine-irrelevant specificities.

Although DC-based synthetic peptide vaccines have not yet been implemented for the treatment of patients with RCC, DC pulsed with alternate formats of RCC-derived antigens have been assessed as vaccines in vitro and in vivo *(159–162)*. In vitro studies performed by Mulders et al. *(159)* used autologous DC loaded with autologous RCC lysates in the presence of liposomes as stimulator cells to promote the expansion of anti-RCC reactive CD4+ and CD8+ TIL. We have used a similar approach to expand anti-RCC reactive CD4+ and CD8+ T cells from the peripheral blood of normal donors (Fig. 2). Thurner et al. *(160)* and Holtl et al. *(161,162)* have initiated a phase I clinical trial in patients with RCC in which autologous dendritic cells derived from monocytes were pulsed with autologous RCC lysate and with the "helper" antigen keyhole limpet hemocyanin (KLH).

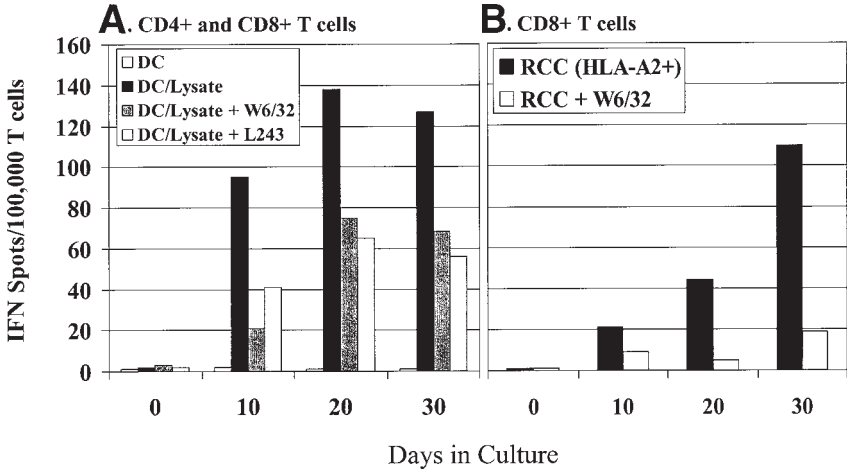

Fig. 2. RCC lysate-pulsed mature DC promote the rapid expansion of RCC-reactive T cells from a normal HLA-A2+ donor. DC were generated from plastic-adherent monocytes obtained from the peripheral blood of a healthy HLA-A2+ normal donor after 5-d culture in serum-free medium containing rhIL-4 + rhGM-CSF. DC were pulsed with freeze-thaw lysate isolated from an HLA-A2- negative RCC cell line and allowed to uptake and process RCC-derived proteins overnight in the presence of maturational stimuli (i.e., rhIL-1β +rhIL-6 + rhTNF-α + PGE2). These antigen-loaded DC were then used to stimulate and restimulate autologous T cells on a weekly basis. On days 0, 10, 20, and 30, T cells were analyzed for their ability to recognize lysate-pulsed autologous HLA-A2+ DC (panel A) or an HLA-A2+ RCC cell line (panel B), in the absence or presence of blocking anti-class I (W6/32) or class II (L243) MAbs, using IFN-γ ELISPOT assays. As indicated, a time-dependent increase in anti-RCC reactivity was noted for both CD4+ (blocked by MAb L243) and CD8+ (blocked by MAb W6/32), with frequencies of both effector cell types exceeding 1/1000 after 3 in vitro stimulations (i.e., at day 30). Results are reported as IFN-γ spots/10^5 T cells plated.

Once matured into CD83+ DC by culture in the presence of TNF-α and PGE_2, these APCs were then injected intraveneoulsy into the autologous patient on a monthly vaccination protocol. Significant DTH reactivity was noted against subcutaneously administered KLH or tumor lysate, supporting the effective vaccine-induction of Th1-type CD4+ T-cell responses *in situ*. Similarly, in vitro analyses of anti-RCC specific T-cell reactivity demonstrated significant vaccine-induced proliferative and cytolytic immune responses. Humoral anti-KLH (IgG + IgM) and anti-RCC lysate (only IgM) were also observed. Of significant note, only minimal toxicity (fever) was associated with these vaccinations. Overall, these initial trials in RCC, combined with the experience gained in melanoma, lymphoma, and prostate carcinoma *(151–153)*, suggests a prototype model of DC-based vaccination (Fig. 3).

The clinical application of DC has prompted a certain degree of caution among some investigators concerned with potential autoimmunity that might occur as a result of using large numbers of such immunostimulatory APC. However, pathologic autoimmunity has not been observed to date in an ever-increasing number of DC-based vaccines *(163)*. Furthermore, based on the findings that most defined TAA and their derivative epitopes are nonmutated, "self" sequences, by definition, those individuals most likely to respond successfully to immunotherapies based on such antigens are precisely those that exhibit the greatest degree of "autoimmunity." Indeed, such a correlation has been noted for RCC patients that display clinical responses to immunotherapy *(164)*. Specifically, those patients that generated enhanced serum titers of autoantibodies directed against thyroglobulin

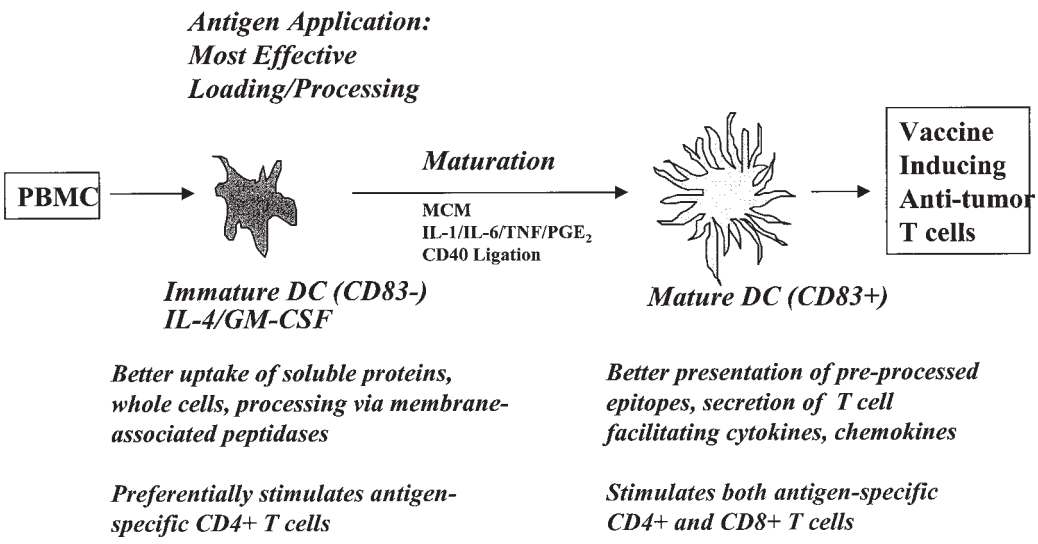

Fig. 3. Schema for DC-based vaccination of patients with RCC. Autologous DC are generated from monocytes as indicated in Figure 2 legend. "Immature" CD83– DC are harvested at day 5–7 of culture. These DC are optimally capable of acquiring exogenous tumor antigens (peptides, proteins, lysate, apoptotic bodies) and may be optimally induced to express both MHC class I- and class II-presented tumor antigen-derived epitopes (with increased MHC-peptide complex stability, Ref. *162*) upon concomitant maturation. Antigen-loaded "mature" CD83+ DC may then be injected (intravenously, subcutaneously, or intralymphatically) into the autologous patient with RCC as a vaccine/immunotherapy. Mature (but not immature) DC express the CCR7 chemokine receptor which promotes directed trafficking to lymphoid tissue in response to MIP-3β *(181)*.

and thyroid microsomes exhibited prolonged survival in response to systemic administration of IL-2 or IFN-α *(164)*.

9. FUTURE DIRECTIONS

Many limitations must be overcome in order to foster optimal efficacy of anti-RCC T cells in vivo, including, identifying means by which to (1) increase antigen-specific CD4+ "helper" and CD8+ CTL numbers and function; (2) target these T cells to tumor lesions; (3) maintain T-cell viability and function in the immunosuppressive tumor microenvironment (for instance, *Fas* expression has been observed in approximately one-third of resected RCC specimens *(165)* presumably resulting in enhanced T-cell apoptosis *in situ [166]*); (4) promote increased infiltration of tumors by additional immune cells (i.e., NK cells, dendritic cells, macrophages, PMN); (5) promote durable immunity that precludes tumor recurrence in patients that display objective clinical responses to immunotherapy. Clearly, Th1-type CD4+ T-cell responses may directly impact each of these issues by potentiating both the afferent and efferent aspects of CD8+ T-cell function, and by mediating DTH reactions within tumor sites, thereby promoting proinflammatory cytokine and/or chemokine production *(86,167,168)*. Cytokines (i.e., IL-2, IFN-γ) may enhance locoregional vascular permeability and increase cellular expression of MHC-peptide complexes, thus, enhancing T-cell reactivity. Chemokines (i.e., IFN-γ dependent IP-10 and *Mig*) may facilitate lymphocyte infiltration and promote the demise of the tumor- associated neovascular bed *(169)*, resulting in tumor necrosis/apoptosis and subsequent acquisition of tumor antigens by DC leading to T-cell induction (Fig. 1).

Whereas the initial focus of RCC vaccines in phase I/II clinical trials is to target the induction and expansion of RCC-reactive CD4+ and CD8+ T cells, subsequent clinical trials must be designed to address these alternate conceptual limitations in order to optimize the clinical potential of such immunotherapies. This may be accomplished using combined immunotherapeutic approaches, such as DC vaccination followed by systemic cytokine application (i.e., IL-2, IL-7, IL-12, IL-15) to enhance tumor antigen-specific T-cell expansion, migration into tumors (vascular leak), and protection from tumor-induced apoptosis (i.e., IL-2, IL-7, IL-12, and IL-15 are antiapoptotic; Refs. *170–173*). Alternatively, (or in conjunction with such approaches) cytokines that promote biased Th1-type immunity (i.e., IL-12, IL-18, IFN-α; Refs. *173–176*) may be targeted to tumor lesions using gene therapy approaches.

Of significant current and future interest will be the application of therapies consisting of tumor-associated antigen cDNA transfected DC. Such gene-modified APCs will allow for a diverse series of tumor antigenic epitopes to be coordinately presented on each of the patient's MHC allelic complexes and can be applied to all patients, regardless of their HLA typing. Initial studies by Alijagic et al. *(177)* supports the ability of human DC transfected with cDNA encoding tyrosinase to promote antimelanoma CTL in vitro. We have similarly demonstrated the ability of DC genetically engineered to express tumor- or viral-associated gene products to promote the induction and expansion of antitumor CTL in vitro and in vivo, with CTL induction enhanced by the cotransfection of DC with cDNA encoding Th1-type biasing cytokines, such as IL-12 or IFN-α *(178–180)*. It would also be conceivable to inject ex vivo-generated DC or cytokine-engineered DC directly into tumors in order to allow for uptake of the full repertoire of RCC-associated antigens (derived from tumor debris or apoptotic bodies) and the subsequent transfer of this immunogenic material to draining lymph nodes, allowing for the promotion of anti-RCC effector T cells.

Although RCC antigen cDNA +/- cytokine cDNA transfected DC may be primarily envisioned as a vaccine, the ex vivo expansion of highly tumor-reactive T cells derived from patient peripheral blood or lymphoid tissue using DC-based protocols for subsequent adoptive transfer into high-risk patients may also represent a tenable therapeutic option. Whereas immunotherapies consisting of the adoptive transfer of autologous TIL or LAK cells plus IL-2 have proven effective in mediating the regression of a minority of patients with advanced metastatic RCC *(27, 29)*, the ex vivo application of DC pulsed with RCC-derived peptides, protein, or cDNA as stimulator cells would be envisioned to yield higher frequencies of therapeutically relevant anti-RCC T cells for adoptive transfer, hence providing theoretically greater clinical benefit.

10. SUMMARY

The past decade has seen the rapid molecular definition of a large number of TAA recognized by T cells, some of which are expressed by RCC. These TAA serve as a logical starting point for the development of well-defined vaccines targeting the induction of antigen-specific cellular immunity and the effective therapy of not only established metastatic disease, but also individuals surgically cured of disease and at high risk for recurrence or development of disease. Despite theoretical concerns of pathologic autoimmunity arising from vaccines constructed from TAA representing (nonmutated) "self" proteins, such toxicity has not been observed in phase I clinical trials to date. Indeed,

limited autoimmunity may represent a positive prognostic factor in defining those patients most responsive to such vaccines. The ideal tumor vaccine designed to effect the systemic eradication of disseminated micrometastatic disease will likely involve the implementation of multiple TAA antigens and epitopes to circumvent immune evasion by evolving RCC placed under immune selective pressure. The phenomenon of "epitope spreading" will also likely serve to temporally expand the repertoire of tumor-associated epitopes recognized by immune-T-effector cells. Further, the immunogenicity of such TAA proteins/peptides will most likely be significantly augmented by the inclusion of state-of-the-art adjuvants geared towards the production of Th1-associated cellular immunity or by the addition of TAA "helper" epitopes. With this knowledge as a foundation, we are now in a favorable position to rationally develop and monitor the clinical impact of vaccines and immunotherapies designed to promote RCC-reactive immunity.

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4 Molecular Mechanisms of Immune Dysfunction in Renal Cell Carcinoma

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Contents

1. INTRODUCTION

The development of an effective host immune response against neoplastic cells requires activation of T cells following recognition of tumor-associated antigens expressed on the appropriate antigen presenting cells *(1)*. The generation of a cell-mediated cellular response typically involves $CD8^+$ T cells that recognize peptides presented by MHC class I whereas $CD4^+$ T cells recognize peptides presented by major histocompatibility complex (MHC) class II along with the appropriate costimulatory molecules (B7.1 and B7.2). Activation of the Th1 $CD4^+$ cells leads to the production of cytokines such as interleukin-2 (IL-2) that provides a critical signal for clonal expansion of antigen activated lymphocytes. IL-2 signaling also upregulates expression of effector molecules (granzyme B and pore forming protein) requisite for the cytolytic function of $CD8^+$ T cells. Another critical cytokine is gamma-interferon (IFNγ), produced by both Th1 $CD4^+$ and a subset of $CD8^+$

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T cells, that further promotes the development of a cellular response by enhancing MHC and costimulatory molecule expression as well as by activating macrophages. Activation of T-cell immunity is dependent on normal intracellular signaling through the T-cell receptor and subsequent downstream induction of a variety of transcriptional factors that regulate gene expression of cytokines, chemokines, and receptors involved in T-cell responses *(1,2)*.

Evidence is accumulating that many types of human tumors have the potential to stimulate a cell-mediated response by virtue of the fact they express tumor-associated antigens recognized by T cells. The most compelling data comes from human melanoma where a number of distinct antigens have been defined, including Mage and MART *(3)*. There is also evidence demonstrating expression of tumor-associated antigens on renal cell carcinoma (RCC) (*4*, Chapter 3). Moreover, T cells isolated and expanded in vitro from a number of different tumor types including RCC have been shown to either secrete cytokines like IFNγ in response to autologous tumor or to mediate tumor specific cytotoxicity *(5,6)*.

Based on these findings, various forms of immunotherapy targeting T cells for activation and expansion have been and are currently under investigation. Patients with RCC and melanoma have been a major focus since early studies with cytokine therapy suggest these tumors were among the most sensitive to immunotherapy *(7,8)*. However, despite encouraging responses in a minor subset of patients, the overall response rate to immunotherapy in kidney cancer remains low irrespective of the approach utilized *(7,8)*. Emerging data suggest that impairment in T-cell recognition of RCC and tumor-induced immune dysfunction in patients lymphocytes may represent barriers to immunotherapy *(9–13)*. In this chapter, we summarize current findings on immune dysregulation in RCC patient T cells and potential mechanisms utilized by tumor cells to evade the immune system and promote their own growth.

2. EVIDENCE FOR IMMUNE DYSFUNCTION IN T CELLS FROM PATIENTS WITH RENAL CELL CARCINOMA (RCC)

Within RCC, there is typically a significant infiltrate of mononuclear cells consisting primary of T lymphocytes and variable expression of macrophages with little accumulation of NK, B cells, or granulocytes *(14)*. The T-cell population is composed of $CD4^+$ and $CD8^+$ subsets containing clones capable of preferentially recognizing RCC as well as nonspecific effector cells *(5,6)*. However, despite this infiltrate, there is no compelling data to suggest the development of a Th 1 type response in the tumor bed. Analysis of cytokine gene expression by reverse transcriptase-polymerase chain reaction indicates variable but typically low percentage of infiltrating lymphocytes that express mRNA for IL-2 and IFNγ *(15,16)*. These findings are similar to those reported for other tumor types *(17)*. Moreover, less than 5% of tumor infiltrating lymphocytes (TILs) express IL-2Rα (mRNA or surface protein) which is upregulated on activated T cells *(12)*. The majority of TIL do express CD45RO, which is a marker of primed T cells, and are depleted of the CD45RA naive population. However, this shift in phenotype from that observed in the peripheral blood is likely a reflection of differences in tissue distribution between $CD45RO^+$ and $CD45RA^+$ population rather than a predominance of functionally activated T cells in the tumor environment *(12,18,19)*.

There is also evidence that freshly isolated TIL demonstrate a selective unresponsive state in vitro suggesting a defect in immunological competence in vivo. TIL from RCC are impaired in their proliferative capacity irrespective of the stimuli employed, which is a

common feature of infiltrating cells from a variety of tumor types *(12,13,20)*. Defective proliferation may be partly linked to altered signaling through the IL-2 receptor (IL-2R). The addition of exogenous IL-2 to in vitro activated TIL expressing IL-2Rα and IL-2Rβ chains did not result in cell cycle progression from G0 through G1 (Kolenko and Finke, unpublished data). This possibility is further supported by the finding that induction of transferrin receptor expression (protein and mRNA), regulated by IL-2 binding to IL-2R, is defective in TIL, but not in peripheral blood T cells of RCC patients *(21)*. The inducible cytolytic capacity of TIL is also diminished. After in vitro stimulation with anti-CD3/IL-2, the non-MHC restricted lytic activity of $CD8^+$ TIL was depressed when compared to that of autologous T cells from the blood and from normal volunteers *(22)*. Poor induction of granzyme B mRNA in approximately half of the TIL examined may contribute to the depressed lytic response because granzyme B plays a significant role in the cytotoxic process *(22)*. The functional deficits in TIL appear to be selective because there is upregulation of IL-2Rα expression following in vitro activation. Furthermore, production of IL-2 and IFNγ by stimulated TIL is similar to that of peripheral blood T cells, suggesting that not all signaling pathways are impaired *(12)*.

There is also evidence for immune dysfunction in vivo. The analysis of delayed type hypersensitivity of RCC patients using common recall antigens (PPD, mumps, and Candida) revealed an impaired response *(23)*. Furthermore, the response rate to PPD skin testing with stage I RCC was significantly higher than that of patients with stage IV disease *(24)*.

3. ALTERED SIGNAL TRANSDUCTION IN CANCER PATIENT T CELLS

Impairment in signal transduction pathways may contribute to the immune dysfunction noted in T cells from the tumor bearing host. Decreased expression of signaling elements linked to T-cell receptor (TCR) have been reported to be depressed in certain murine tumor models and in patients with cancer *(25–28)*. This includes the ζ chain of the TCR as well as the expression of associated tyrosine kinases, $p56^{lck}$, $p59^{fyn}$, and ZAP-70 *(25–28)*. Expression of the ζ chain has been the most thoroughly studied and has been reported to be depressed in T cells from a number of histological types of human tumors *(27–31)*. The most pronounced changes in ζ chain expression are seen in tumor infiltrating lymphocytes although reduced levels are also noted in peripheral blood T cells *(28–30)*. In some tumor types such as melanoma, cervical cancer, and head and neck tumors, reduced expression of the ζ chain is associated with impaired cyokine production (IL-2, TNFα, and IFNγ) by peripheral blood T cells as well as with poor clinical outcome *(28, 29,31)*. Several different mechanisms have been proposed to account for the defect in ζ chain expression. Two different groups showed that hydrogen peroxide produced by tumor-associated macrophages downregulates ζ chain expression and inhibits tumor specific T-cell and natural killer cell-mediated cytotoxicity *(32,33)*. Chronic antigen stimulation has been suggested as another means of down-regulating the ζ chain *(34)*. Additional data suggest that the ζ chain is degraded by activate caspases 3 and 7 in T cells as a consequence of apoptosis *(35)*. In RCC, there is controversy over whether the ζ chain is altered because findings ranging from no suppression to variable decreases in protein expression have been reported *(13,36,37)*. In addition studies from our laboratory could not find any correlation between the decreased expression of the ζ chain in a subset of patients and various clinical parameters *(38)*. Thus, the contribution that impaired expression of the ζ chain makes to the immune dysfunction in RCC patients is not well defined.

Table 1
Impaired NFκB Activation in Patient T Cells

Patient Population	*% of Patients Defective NFκB*
Normal	6 (53)[d]
NED Stage III/IV[a]	17 (18)
Metastatic[b]	69 (42)
Localized[c]	63 (46)
T-Cell Source	*% of Patients with Normal κB Binding*
Localized Disease	35 (6/17)[e]
Postnephrectomy	
In vitro cell culture	58 (7/12)

[a] Control Normals vs NED $p = 0.33$.
[b] Control Normals vs Metastatic $p < 0.0001$.
[c] Control Normals vs Localized $p < 0.0001$.
[d] Number of RCC Patients.

There is also evidence that activation of the transcription factor NFκB is impaired in T cells from tumor bearing host that may contribute to the immune dysfunction *(39–41)*. The nuclear translocation of NFκB regulates the transcription of genes involved in the development of T-cell immunity. NFκB consists of multiple members of the Rel family of proteins that include NFκB1 (*p*105/*p*50), NFκB2 (*p*l00/*p*52), RelA (*p*65), and c-Rel *(42–44)*. Rel proteins form hetero- and homodimers which differ in their transactivating activity *(42–44)*. In T lymphocytes, the RelA/*p*50 heterodimer is known to initiate transactivation while homodimers of *p*50 are thought to be inhibitory *(42–44)*. NFκB dimers are retained in cytoplasm in an inactive form bound to inhibitory proteins, IκB (e.g., IκBα, IκBβ, and IκBε) *(42–44)*. Following stimulation, IκBα is phosphorylated, which marks the inhibitor for ubiquitination and degradation by the proteasome-dependent pathway *(42–44)*. This process allows nuclear localization of NFκB complexes where they bind to specific DNA motifs and activate transcription *(42–44)*.

Animal studies have documented impairment in NFκB translocation during tumor progression which appears to precede the alteration in the ζ chain expression of T cells *(39,40)*. Moreover, the decrease in NFκB activation coincided with reduced expression of IL-2 and IFNγ production *(39,40)*. We have demonstrated impaired activation of NFκB in T cells derived from RCC patients *(41)*. The major problem is the normal nuclear accumulation of NFκB after activation *(45)*. The cytoplasmic levels of NFκB proteins are not altered. Several lines of evidence suggest that suppression of NFκB maybe mediated by the tumor *(46)*. This defect was noted in the majority of TIL samples tested and in peripheral blood T cells from 60% of RCC patients (n =88) (Table 1). In contrast, this defect was only observed in 6% of T cells (n = 53) from normal healthy volunteers. Of interest was the fact that this defect was only present in 17% of (n =18) patients with advanced disease but with no evidence of disease (NED) following surgical treatment. Further, this defect was reversible in vivo *(46)*. In patients with localized disease who had impaired NFκB activation prior to surgery, 35% (n=17) showed normal κB binding activity within 8 wk of complete tumor removal. In addition, the culturing

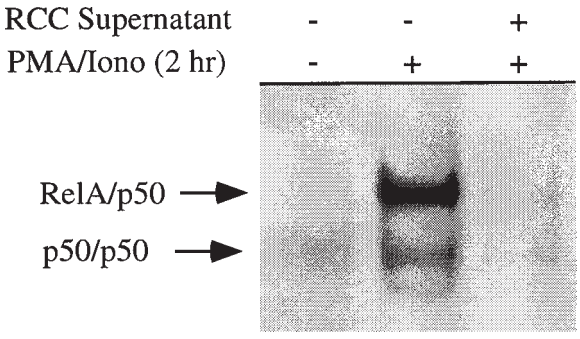

Fig. 1. Supernatants from RCC can suppress NFκB activation in normal T cells. T cells from a healthy donor were incubated for 18 h with media or media supplemented with RCC supernatant (50% volume). Thereafter, cells were stimulated with PMA/ionomycin for 2 h prior to performing EMSA on nuclear extracts.

of patients T cells in medium alone for 72 h resulted in normal κB binding activity in 58% of the experiments ($n = 12$). It has also been demonstrated that soluble products derived from cultures of renal tumor explants can suppress NFκB activation in T cells from normal volunteers (Fig. 1). Supernatant from uninvolved kidney had minimal effect.

Recent findings demonstrate that two distinct mechanisms are responsible for the defect in NFκB activity in RCC patient T cells and each may be mediated by a different tumor-derived product *(45)*. In one subset of RCC patients, impaired κB binding activity results from retention of NFκB dimers in the cytoplasm because there is no stimulus dependent phosphorylation and degradation of the inhibitor, IκBα *(45)*. The responsible product appears to be a low molecular weight molecule that is sensitive to proteinase K treatment (Finke, unpublished data). In another set of patients, impaired κB binding activity occurs in the presence of normal degradation of IκBα. In these patients, poor accumulation of Rel dimers in the nucleus may be attributed to degradation of Rel proteins by nuclear proteases. Gangliosides shed from RCC may be responsible for this defect in NFκB activation (Uzzo, et al., *J. Clin. Invest.,* in press).

An important issue to address is what are the functional consequences of impaired NFκB binding activity for patient T cells. Our findings suggest that this defect in NFκB may make T cells more susceptible to apoptosis. There is growing evidence that NFκB regulates the susceptibility of certain cell types to apoptosis through the transcriptional control of protective genes *(42–43)*. Knockout transgenic mice lacking the RelA component of NFκB complex displayed embryonic lethality and liver cell apoptosis *(42–43)*. Inhibition of NFκB nuclear translocation also enhanced apoptosis induced by TNFα, ionizing radiation or the chemotherapeutic drug, danorubicin *(42,43,47)*. NFκB also appears to regulate the susceptibility of lymphoid cells to apoptosis. Addition of various inhibitors of NFκB/Rel activation to normal murine B lymphocytes or to B-cell lymphomas resulted in apoptosis *(48)*. There are several lines of evidence to suggest that alteration in NFκB activation in RCC patient T cells increases their sensitivity to apoptosis. We have observed T cells in the tumor bed with evidence of DNA breaks as defined by the TUNEL assay *(49)*. In addition, peripheral blood T cells from 50% of RCC patients, but not normal individuals, display an early marker of apoptosis, the externalization of phosphatidyl serine *(49)*. Finally, we have shown in normal T cells that the suppression of NFκB activation by a cell permeable peptide, SN50 *(50)* can induce apoptosis and

make cells more susceptible to apoptosis following treatment with anti-Fas antibody (Finke, unpublished).

4. SOLUBLE PRODUCTS PRODUCED BY RCC MAY INHIBIT T-CELL ANTITUMOR ACTIVITY

The process of malignant cellular transformation confers a selective survival advantage. Neoplastic cells are known to produce a variety of biologically active substances that may contribute to their proliferation and survival, either directly by autocrine or paracrine effects on transformed cells, or indirectly by muting the normal immune cellular response to foreign cellular proteins. A cell that has been transformed may, therefore, continue to produce substances it generated before oncogenesis, albeit in a manner which may differ quantitatively or qualitatively. In addition, new or mutated cellular proteins, such as tumor suppressor gene products, may also contribute to the unregulated growth that is characteristic of transformed cells.

Inadequate function of T-TILs may be attributable in part to a variety of immunosuppressive molecules produced by the tumor and/or its associated stroma. These products, including cytokines such as IL-10 and transforming growth factor-β (TGF-β), prostaglandins and tumor-derived gangliosides, may serve as paracrine mediators to diminish effective cellular antitumor immunity via their effects on T-cell surface molecules, signaling events, and ultimately effector function.

The complex relationship between cytokines and cancer is poorly understood. Under optimal circumstances, local production of cytokines and chemokines within the tumor bed would result in recruitment and activation of tumor-associated inflammatory cells capable of initiating an effective antitumor response *(51)*. However, there is little evidence for this type of effective orchestrated cytokine activity within the bed of most solid tumors. Instead, the predominant cytokine expressed in the tumor including RCC appears to be the Th2 cytokine IL-10 *(15,16)*. The biologic properties of IL-10 may counteract both specific and nonspecific T-cell mediated immunity, primarily because of its ability to downregulate MHC class II expression on monocytes/dendritic cells leading to impaired antigen presentation *(52)*. IL-10 also has direct inhibitory effects on T-cell growth *(53)*, which may be related to its known ability to inhibit the transcription factor NFκB *(54)*, as well as an antiinflammatory effect by suppressing the release of monocyte derived reactive oxygen intermediates *(55)*. As noted previously, NFκB participates in the transcriptional control of a diverse set of genes whose products play an important role in T-cell activation and the development of cellular immunity *(42–44)*. These include cytokines and their receptor genes, interferons, growth factors, and other immunoregulatory and leukocyte adhesion molecules *(42–44)*. Suppression of NFκB may represent one mechanism whereby IL-10 can reduce the production of Th1 cytokines *(56)*. IL-10 can further reduce antitumor immunity by suppressing the secretion of other pro-inflammatory cytokines such as IL-1, IL-6, IL-8, and TNF-α *(57)*. Production of IL-10 has been attributed to both primary tumor cells *(58)* as well as inflammatory cells such as activated monocytes and helper T cells *(15,16)*. The relative contribution of each of these sources of IL-10 within the tumor bed and its implication for alterations in T-cell activity remain to be elucidated. However, recent findings suggest that IL-10 may play a role in potentiating tumor progression in kidney cancer, since elevated pretreatment serum levels of IL-10 was an independent predictor of unfavorable clinical outcome *(59)*.

A second immunosuppressive cytokine that may reduce effective T-cell function in RCC is TGF-β. Expression of TGFβ1 by primary RCC cell lines is well documented *(60, 61)* with increased levels identified in the serum of patients with this disease *(62,63)*. Although TGF-β has been demonstrated by some to inhibit the growth of RCC cell lines in vitro *(64,65)* its primary effect in vivo may be as a potent inhibitor of IL-2-dependent T-cell proliferation *(66)*. TGF-β inhibits lymphocyte proliferation at very low (femtomolar) concentrations suggesting that it is more potent than even the T-cell specific immunosuppressant cyclosporin A in diminishing the T-cell response *(66,67)*. Such low levels of TGF-β are almost certainly attainable within the tumor microenvironment. The primary effect of TGF-β appears to be reduction of T-cell proliferation by inhibiting both TCR and IL-2R mediated tyrosine phosphorylation, which affects subsequent downstream signaling events central to the control of cell cycle progression such as phosphorylation of the Rb protein *(66)*. These antiproliferative effects appear to involve both helper and cytotoxic T cells *(68)*.

Prostaglandin E_2 (PGE_2) is also known to inhibit T-cell activation *(69,70)* and may functionally diminish immune cellular responsiveness in the tumor bed of patients with RCC. The mechanism of T-cell suppression by PGE_2 appears to be through increasing the levels of the intracellular second messenger, cyclic adenosine monophosphate (cAMP) *(70)*. PGE_2 is also known to inhibit the DNA-binding activity of the transcriptional factor, NFκB to the IL-2 transcriptional start site, thereby blocking production of IL-2 *(71)*. More recent studies indicate that PGE_2-induced inhibition of T-cell proliferation is mediated through blocking IL-2-dependent G1-S transition *(72)*. This defect may be because of downregulation of JAK3 expression resulting in impaired phosphorylation and DNA binding activity of STAT5 (signal transducer and activator of transcription) *(73)*. Moreover, NK cell function may also be suppressed by prostaglandins present within the tumor environment. Macrophages from Renca-bearing mice were found to suppress the generation of LAK and NK cells in vitro by synthesizing prostaglandins. Indomethacin, a prostaglandin synthetase inhibitor, blocked the induction of suppression both in vitro and in vivo, suggesting the presence and biological significance of endogenous prostaglandins in Renca-bearing animal models *(74)*. Prostaglandins may contribute to the immune dysfunction in patients with kidney cancer. Increased levels of PGE_2 have been found in the venous effluent of kidneys affected with RCC, supernatant from RCC explants, and from primary RCC cell cultures (*75*, Finke et al., unpublished observation). Furthermore, we have shown that supernatant fluids from RCC containing PGE_2 can suppress IL-2R signaling and T-cell proliferation. The mechanism of suppression may be cAMP dependent, because the signaling events inhibited by RCC supernatant are similar to those suppressed by agents that increase cAMP (i.e., forskolin) and include JAK3 expression, IL-2R-linked STAT5 translocation, and expression of c-Myc and c-Jun *(73)*.

A third important class of soluble mediators that may inhibit the host immune response is tumor-derived gangliosides. These glycosphingolipids are overexpressed in several tumor types including RCC *(76–78)* and may inhibit several steps critical to effective cellular immunity including antigen presentation or processing *(79)*, lymphocyte clonal expansion *(80)*, and cytotoxic effector function *(81)*. As components of the tumor cellular membrane, they are thought to be at highest concentrations in the tumor microenvironment but may be shed into the circulation in the form of monomers, micelles, or membrane vesicles *(82)*. Shed tumor gangliosides are amphipathic and capable of incorporating into the cellular membrane of lymphoid cells, thereby altering the growth behavior of the cell

(76). They have been shown to inhibit murine allogeneic cellular responses in vivo as well as human proliferative T-cell responses in vitro *(83)*. Furthermore, membrane bound gangliosides may modulate transmembrane signal transduction via alterations in kinase activity associated with growth factor receptors and protein kinase C *(84–87)*. Purified bovine brain gangliosides have also been shown to suppress NFκB specific binding activity in T cells and inhibit transcription of IL-2 and IFNγ without affecting IL-4 or IL-10 production, suggesting they may contribute to a functional Th1 to Th2 cytokine shift *(88)*. These same defects have been shown to exist in peripheral blood and tumor infiltrating T cells from patients with RCC and can be mimicked in T cells from healthy individuals by soluble products present in RCC tumor supernatant (Uzzo, et al., *J. Clin. Invest.,* in press). We have recently identified tumor-derived gangliosides as one metabolically active component of crude tumor supernatant from RCC explants. Gangliosides have been isolated from RCC tumor and supernatant, but not from that of adjacent normal kidney, and are capable of inhibiting NFκB activation in normal T cells. The suppression of κB binding activity occurred in the setting of normal upstream signaling events such as degradation of the cytoplasmic inhibitor of NFκB activation, IκBα.

Soluble products from renal cell carcinoma may, therefore, diminish effective antitumor immunity through a number of mechanisms including decreased T-cell proliferation or cytokine expression that results from select alterations in signal transduction pathways critical for normal T-cell activation. Important recent evidence suggests that gangliosides and other soluble mediators may also be required for Fas (CD95) mediated apoptosis in human T-cell lines *(89)*. Induction of apoptosis in T cells as a mechanism for immune suppression is further discussed below.

5. REDUCED EXPRESSION OF ANTIGEN PRESENTATION MACHINERY IN RCC CONTRIBUTES TO IMMUNE DYSFUNCTION

Recently, the molecular basis of recognition of tumor antigens by cytotoxic T cells (CTL) has become better elucidated. CTL directed against autologous RCC have been generated, including MHC-restricted $CD8^+$ lines *(5,6,90,91)*. However, before CTL recognition of discrete RCC tumor antigens can result in effective antitumor activity in vivo, there must first be efficient processing of antigenic peptides and presentation in a MHC class I-restricted manner by the tumor cells or dedicated antigen presenting cells (APCs) to appropriate effector T cells. Downregulation of components of antigen processing and presenting machinery has been identified in human malignant cells including RCC, which may blunt endogenous antitumor immunity *(92)*. This defect includes reduced or absent expression of MHC class I heavy and light chains, diminished levels of TAP (transporters associated with antigen processing) proteins, as well as deficient expression of LMP proteosomal complexes *(93)*. Tumors and other epithelial cells have the potential to present foreign antigen on their surface to T cells; however, these peptides must first be processed and loaded into the MHC class I binding cleft. Foreign peptides are, therefore, cleaved in the cytoplasm of APCs by proteosomal subunits including LMP-2 and -7. These peptide fragments then associate with TAP in the endoplasmic reticulum where they are loaded onto MHC class I molecules prior to expression on the cell surface *(92)*. In addition to RCC, downregulation of the components of cellular antigen processing and presentation have been identified in a number of other solid tumors, including lesions of the lung, liver, prostate, colon, cervix, skin, and breast. Thus, TAP

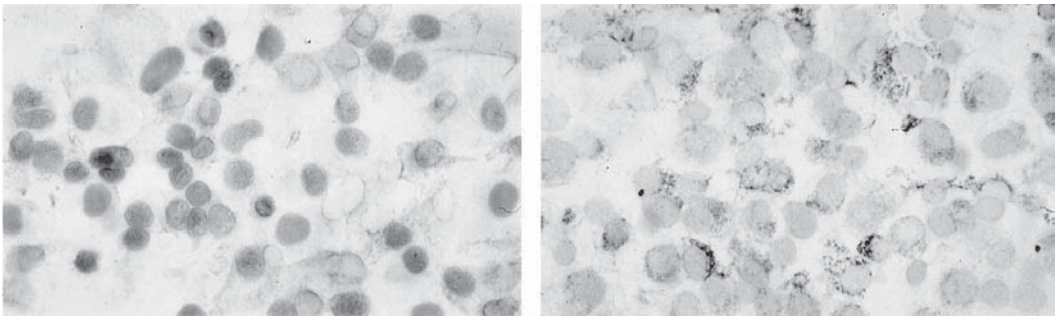

Fig. 2. RCC cell lines express *Fas*-L as defined by immunostaining of cytospins. *FasL* expression was detected by immunoperoxidase staining using anti-*FasL* antibody (Transduction Lab) (right panel). Immunostaining with control antibody did not show any positive reaction (left panel).

and MHC defects may provide malignant cells with a mechanism for escaping CTL-mediated recognition and destruction. The reduction in mRNA and protein levels of these molecules appears more pronounced in RCC cells that have acquired metastatic potential suggesting that the process of malignant transformation may include progressive loss of these functionally important molecules *(93)*. Increasing the levels of TAP in RCC, either through cytokine treatment with IFN-γ or by transfection with TAP-1 cDNA results in higher expression of MHC class I-molecules on the tumor cell surface, and enhanced tumor specific, class I-restricted CTL recognition *(92,94)*. Understanding and overcoming defective antigen presentation is an important component to any effective immunotherapy strategy.

6. TUMOR INDUCTION OF APOPTOSIS IN T CELL DIMINISHES EFFECTIVE ANTITUMOR IMMUNITY

Recent evidence suggests that cell mediated immunity may be downregulated through apoptotic pathways *(95,96)*. Interactions between the *Fas* receptor (*Apo*-1/CD95) and its ligand (*Fas-L*/CD95-L) have been implicated in a number of normal and pathological processes regulating T-cell function. *Fas*-L is used by lymphocytes not only as a cytotoxic effector mechanism to induce apoptosis in *Fas* expressing targets *(97–99)*, but also to diminish the immune response once the targeted antigen has been eliminated *(100,101)*. *Fas*/*Fas*-L-mediated induction of apoptosis is, therefore, an effective mechanism of T-cell homeostasis whereby self-reactive clones can be eliminated *(100)*, conditions of tolerance *(102)* and immune sanctuaries can be achieved *(103,104)*, and overexuberance of the immune response can be prevented *(105)*. However, tumor cells may take advantage of these mechanisms to escape immune detection and destruction. Malignant cells from an increasing number of solid tumors including RCC *(49)* have been shown to express *Fas*-L and tumor infiltrating lymphocytes (T-TIL) are potential targets for these *Fas*-L expressing tumor cells *(106)*.

We have recently demonstrated the presence of *FasL* on renal tumor cells using a number of different techniques (Fig. 2). mRNA of *FasL* transcripts were identified in short and long term RCC cell lines by RT-PCR. This corresponds to FasL protein expression as measured by Western blotting of whole cell lysates, cytospins of short-term tumor culture, *in situ* immunohistochemistry of fresh fixed tumor specimens, and immunocytometry of tumor cell suspensions *(49)*. T cells derived from the peripheral blood as well

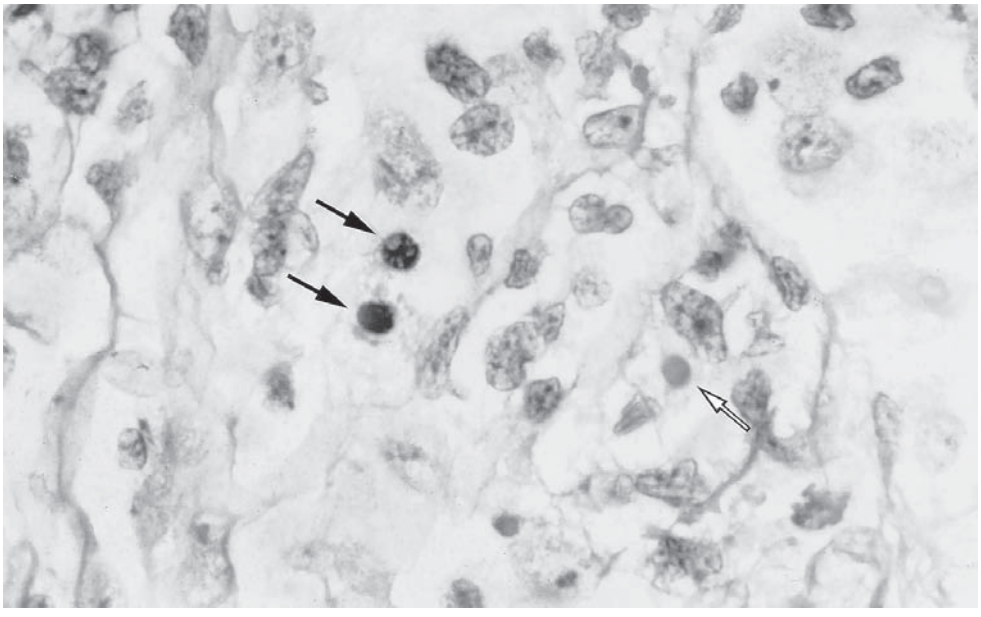

Fig. 3. TUNEL assay demonstrate the presence of T cells with DNA breaks within the tumor bed. *In situ* TUNEL assay was performed on tissue sections from RCC patients (10 different patients, representative data shown). Closed arrows indicate T cells undergoing apoptosis whereas open arrow represent a T cell that does not show any DNA breaks. Double staining with anti-CD3 anitbody confired that the cells examined were indeed T cells.

as those infiltrating the tumor have been shown to express Fas receptor *(49,107)*. These cells are therefore potential targets for apoptosis mediated by FasL expressing tumor. When preactivated allogeneic T cells or Jurkat T-cell line were cocultured with RCC tumors expressing FasL, they exhibit DNA breaks as measured by TUNEL assay. This lethal interaction may be partially blocked by antibodies against FasL, supporting its role in T-cell death. Furthermore, there is clear and consistent evidence for T-cell apoptosis in the tumor bed of RCC malignancies (Fig. 3). Taken together, these data support the role of tumor mediated apoptosis as a mechanism of inhibition of an effective T-cell response to renal tumors.

We have recently identified a second potential mechanism whereby T-cell deletion may occur via apoptosis thereby limiting effective antitumor immunity. The process of activation induced apoptosis (AICD) is critical to the downregulation and termination of a completed immune response. Ordinarily, when stimulated T cells are reactivated, they undergo apoptosis so as to prevent overexuberance of the T-cell response. However, peripheral blood T cells from patients with RCC appear susceptible to AICD upon initial stimulation with various stimuli including the phorbol ester PMA in combination with the calcium ionohore ionomycin, as well as by stimulators of the TCR and TNF-R. This finding suggests that when T cells are stimulated by tumor antigen, they may undergo cell death rather than cell activation. Consistent with these emerging data are the findings of increased constitutive expression of the early apoptotic marker, phosphatidly serine (detected by Annexin V staining) on the surface of T cells isolated from patients with RCC. Indeed, certain subsets of T cells from affected patients exhibit characteristics of a "preapoptotic" state and undergo apoptosis upon stimulation. This may be caused in part

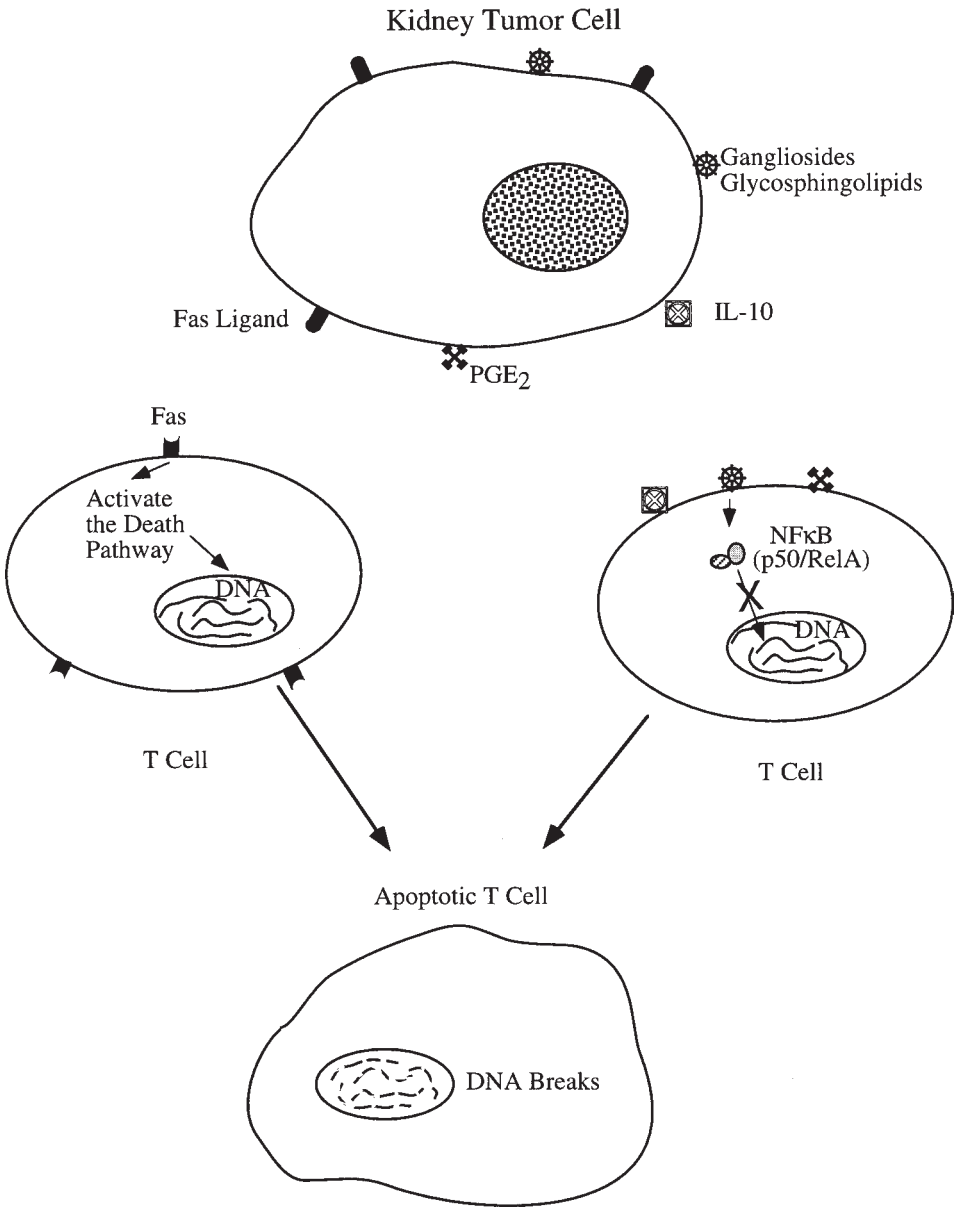

Fig. 4. Scheme of potential mechanism of immune suppression.

to their known alteration in NFκB binding activity, where increasing evidence supports the role of NFκB as a survival factor inhibiting activation induced apoptotsis *(47)*. Thus, as shown in Fig. 4, we suggest that apoptosis of T cells contributes to the immune dysfunction and that this process may be mediated by various tumor derived products directly (*FasL*) or indirectly by suppressing NFκB (gangliosides, and possibly IL-10 and PGE2).

Understanding these mechanisms of defective cellular signaling and their relationship to induction of apoptosis in T cells is critical for designing strategies to overcome tumor induced immune suppression.

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5 Molecular Genetics of Renal Cell Carcinoma

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Contents

1. INTRODUCTION

Renal cancer is diagnosed in about 28,800 Americans each year, and is responsible for more than 11,300 deaths per year *(1)*. Renal cancer is most commonly diagnosed between the age of 50 and 70, and affects men twice as frequently as women *(2)*. Environmental factors linked to the development of renal cancer include cigarette smoking, obesity, and exposure to asbestos, cadmium, or petrochemical products *(3–8)*.

Renal tumors were first called hypernephroma by Grawitz in1883, presuming a relation of these tumors to the adrenal gland located above the kidney *(9)*. Subsequently, renal tumors have been divided into different types based on histologic and morphologic

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Table 1
UICC Histological Classification of Renal Tumors

Benign Renal Tumors
Metanephric adenoma
Adenofibroma
Papillary adenoma
Oncocytoma
Malignant Tumors
Clear cell (conventional) renal carcinoma
Papillary renal carcinoma
Chromophobe renal carcinoma
Collecting duct carcinoma
Unclassified renal cell carcinoma

features. In 1997, a combined European and American work-group adopted a new histologic classification system (Table 1) *(10)*. In this system, 80–85% of renal cancers are of the clear cell type and 5–10% are of the papillary type *(2)*.

The study of hereditary forms of renal cancer has been essential to the understanding of genetic causes of renal cancer. To date, four inherited forms of renal cell carcinoma (RCC) have been described. The most studied form is von Hippel Lindau disease (VHL), an autosomal dominant disorder in which affected individuals can develop hemangioblastoma of the central nervous system (CNS), retinal angiomas, pancreatic neuroendocrine tumors, microcystic adenomas, and cysts, epididymal cystadenomas, as well as clear cell renal cancer *(2,11)*. VHL is clinically caused by mutations in the *VHL* gene located on chromosome 3p *(12)*. A related, but less common, inherited form of clear cell renal cancer is hereditary clear cell renal carcinoma (HCRC). This autosomal dominant inherited disorder is characterized clinically by multiple bilateral clear cell renal tumors and genetically by germline translocations of the short arm of chromosome 3(p) *(13–15)*. A third hereditary renal cancer is hereditary papillary renal carcinoma (HPRC), characterized clinically by basophilic papillary renal tumors and activating germline mutations in the *met* gene *(16–18)*. Hereditary renal oncocytoma (HRO) has recently been characterized clinically *(19)*. In contrast to sporadic renal oncocytoma, these hereditary forms are usually bilateral and multifocal, and occur at a younger age than sporadic renal cancers *(2)*.

2. TUMOR SUPPRESSOR GENES AND ONCOGENES

Two principal types of genetic changes have been associated with tumor formation. Loss of function mutations, thought to occur with tumor suppressor genes, and gain of function mutations, which occur in oncogenes.

The first evidence suggesting that genes existed that could suppress tumor growth came from cell fusion experiments. It was found that the merging of malignant cell lines with normal cells produced hybrid cells that were no longer capable of tumor formation in animals *(20,21)*.

Statistical evaluation of retinoblastoma and Wilms' tumor patients by Knudson and Strong supported familial tumor formation as a single event phenomenon, whereas sporadic tumors developed with at least two events (Fig. 1) *(22,23)*. This single event was hypothesized to be inactivation of a tumor suppressor gene in patients with an inherited mutation

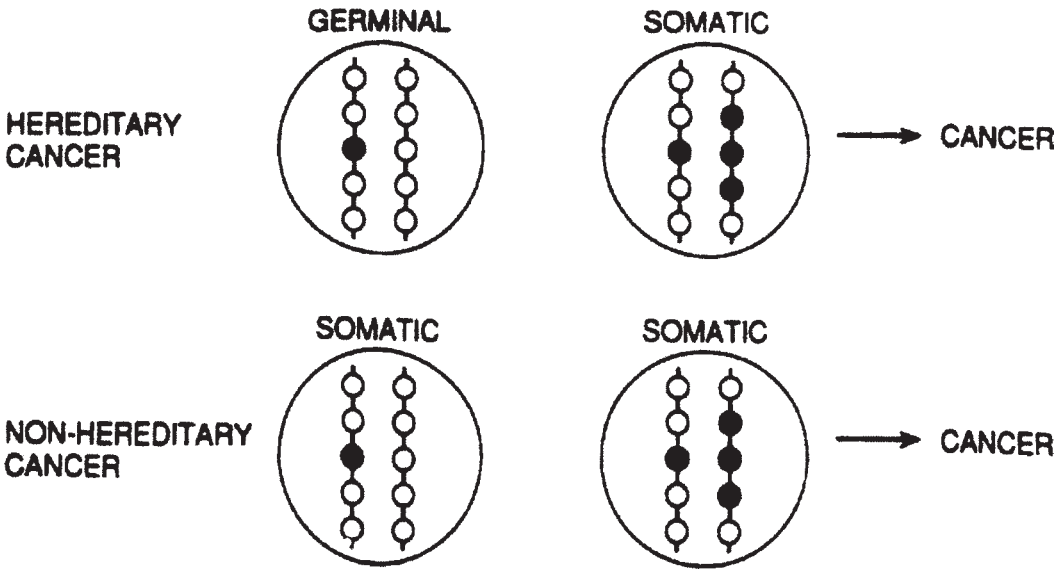

Fig. 1. Early work by Knudson led to the hypothesis that inactivation of both copies of a gene, called a tumor suppressor gene, could lead to tumor formation. In the hereditary renal cancer syndrome VHL, affected members inherit a germline copy of a mutated *VHL* gene from an affected parent. A subsequent somatic *VHL* gene mutation leads to tumor development. In nonhereditary, or sporadic renal cancer, both alleles are inactivated by somatic mutation (from Zbar et al. *[95]*).

in one of their two copies, and was the first human evidence of its presence. The presence of such an inherited gene also helped explain the formation of bilateral, multifocal tumors at an earlier age in affected families, in contrast to patients with sporadic tumors.

Tumor suppressor genes generally regulate cellular growth, and loss of their function leads to loss of cell cycle control. Because there are two copies of each gene, inactivation of both copies is required *(22,23)*. One copy of a tumor suppressor gene is usually lost by deletion of a large DNA fragment, which can be detected as loss of heterozygosity (LOH). The second copy may be inactivated by gene mutation or hypermethylation. A high frequency of LOH at a genetic locus is thus often interpreted as evidence of a tumor suppressor gene.

Protooncogenes are normal genes generally involved in the basic cell functions of cellular growth, differentiation, or proliferation. Mutations of these protooncogenes lead to a gain of function, and are called oncogenes. Oncogene formation may occur by a number of mechanisms including point mutation or fusion with "activating" genes. These changes lead to an increased amount of normal protein or creation of a mutant "turned on" signaling protein, which alters cell growth and results in malignant transformation of the cell *(25)*. In contrast to tumor suppressor genes, oncogenes are not usually associated with LOH.

3. FAMILIAL CLEAR CELL RENAL CANCER

There have been rare reports of families with a hereditary form of conventional renal cancer having germline translocations, [t(3;8) *(26–28)*, t(3;6) *(29)*, and t(3;2) *(30,31)*]. All affected individuals had a balanced translocation between chromosome 3p and a

different chromosome. The breakpoint on chromosome 3 was localized at a fragile breakpoint, 3p13–14.2, in all families. The translocated copy of chromosome 3 was lost in the renal tumors, suggestive of a tumor suppressor gene. The involvement of chromosome 3 in all these families suggested mutations in this gene might be important in the initial development of clear cell RCC. Genetic evaluation of clear cell renal tumors from these families has demonstrated the presence of *VHL* gene mutations *(14,31–33)*.

4. CHROMOSOME 3: LOCATION OF THE GENE FOR CLEAR CELL RENAL CARCINOMA

The findings of chromosome 3p abnormalities in familial forms of clear cell renal cancer led to genetic studies of chromosome 3 in nonhereditary RCC. The genetic findings in the familial setting were suggestive of a tumor suppressor gene, which is associated with loss of heterozygosity in sporadic tumors. Zbar et al. used restriction fragment length polymorphism (RFLP) analysis to evaluate patients with sporadic clear cell renal cancer and found loss of heterozygosity in the 3p14–21 region in 11 of 11 evaluable tumors *(34)*. Using a large panel of chromosome 3p markers, Anglard et al. detected LOH in 88% of 60 clear cell renal tumors, and defined an area of minimal deletion in the 3p21–26 region of chromosome 3 *(35)*. Later evaluation of sporadic clear cell tumors for chromosome 3p LOH by RFLP analysis demonstrated consistent loss in up to 95% of tumor tissue *(36–39)*. In contrast, nonclear cell or papillary renal carcinomas did not have chromosome 3p LOH *(35,40–43)*.

5. VHL DISEASE

VHL is an autosomal dominant inherited cancer syndrome characterized clinically by the development of CNS hemangioblastomas, retinal angiomas, endolymphatic sac tumors, pheochromocytoma, neuroendocrine tumors of the pancreas, epididymal cystadenomas, and clear cell renal cancer *(11)*. The study of a hereditary form of renal cancer was performed to take advantage of the powerful tools of linkage analysis to identify the VHL gene as a candidate clear cell renal cancer tumor suppressor gene.

Between 28–45% of affected VHL patients develop renal cancer, and the mean age at diagnosis is 39 years *(44)*. Affected individuals may develop clear cell and eosinophilic renal cysts, renal cysts lined with clear cell renal cancer, and/or solid clear cell renal cancers *(45)*. Prior to the use of CT imaging, as many as 42% of VHL patients died from metastatic renal cancer *(44,46,47)*. With CT imaging, renal tumors detected by screening-affected kindreds are often small and are confined to the kidney *(48,49)*. The multiple bilateral nature of hereditary kidney cancer is best suited to a parenchymal sparing enucleation technique *(50)*.

6. LOCALIZATION OF THE *VHL* GENE TO CHROMOSOME 3 USING LINKAGE ANALYSIS

Identification of the *VHL* tumor suppressor gene was performed using genetic linkage analysis and positional cloning. Random DNA chromosome markers are studied to determine if they correlate with clinical manifestations of the disease. When these markers do segregate with the disease, they are said to link to the disease. Seizinger et al. evaluated 203 members of nine VHL families, including 71 affected members, and found linkage

to the *RAF1* oncogene chromosome 3p25 *(51)*. Not all affected members linked to this gene, however, indicating the *VHL* gene was not *RAF1*.

To further localize the *VHL* gene, Lerman et al. isolated a collection of 2000 DNA fragments from human chromosome 3 *(52)*. These single-copy DNA fragments were sorted and used to construct a linkage map encompassing the distal portion of chromosome 3p and the VHL locus. Hosoe et al. used this map to study 25 families with VHL *(53)*. Using linkage analysis, the *VHL* gene was localized to a small region on chromosome 3p, between *RAF1* and *D3S18*, a polymorphic DNA marker located at 3p26. The localization of the *VHL* gene to this small region on chromosome 3p25–26 made possible subsequent cloning studies that identified the *VHL* gene.

7. *VHL*, A TUMOR SUPPRESSOR GENE

Knudson's two-hit hypothesis for a tumor suppressor gene predicts both copies of a gene must be inactivated for tumor formation to occur. In the familial setting, the mutated copy of the *VHL* gene inherited from the affected parent would be retained, whereas the normal copy from the unaffected parent would be lost in VHL-related tumors. Tumor tissue from VHL patients was examined for chromosome 3p LOH to test this hypothesis. Tory et al. consistently found loss of the same chromosome 3p allele when multiple tumors were examined from a single patient *(54)*. In each patient, the chromosome 3p allele lost was inherited from the unaffected parent, carrying the normal copy of the *VHL* gene. This report demonstrated inactivation of both copies of the *VHL* gene in tumor tissue, one by inheritance (germline mutation) and one by deletion (loss of somatic allele). These findings are compatible with Knudson's tumor suppressor gene model, and suggest both copies of the *VHL* gene must be inactivated before tumor formation can occur.

8. IDENTIFICATION OF THE *VHL* GENE

Genetic linkage analysis had narrowed the search area for the *VHL* gene to a small region on chromosome 3 (i.e., 3p25), the same region localized by LOH studies in patients with noninherited or sporadic clear cell kidney cancer. To further localize this gene, a contiguous DNA segment map consisting of cloned DNA yeast artificial chromosomes, cosmid and phage contigs were constructed in the 400 kb region thought to harbor the *VHL* gene *(55)*. Pulsefield electrophoresis identified overlapping germline deletions in three unrelated VHL families *(56)*. This critical observation narrowed the search to a particular cosmid in the DNA physical map (ie, cosmid 3, a previously cloned fragment of DNA). Latif et al. subsequently identified two complementary DNAs cDNAs) from this cosmid as candidate *VHL* tumor suppressor genes *(12)*. The g7 cDNA or gene was found to be a strong candidate for the VHL disease locus, because germline DNA rearrangements of *g7* were found in a significant number of affected individuals from VHL families *(12)*. Furthermore, the *g7* gene was found to be expressed by Northern blotting in all human tissues tested, including brain and kidney, tissues frequently affected in VHL disease *(12)*. The predicted gene sequence lacked homology to other known proteins, except for a small region of a surface membrane glycoprotein from *Trypanosoma brucie (12)*. The *g7* gene, or *VHL* gene, was found to be highly conserved across species ranging from mammals to *Drosophila*, consistent with an essential role in life processes. The *VHL* gene identified is small, composed of three exons with an open reading frame of 852 nucleotides and encoding a protein of 284 amino acids (Fig. 2) *(12)*.

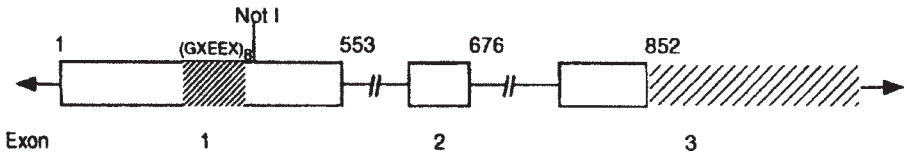

Fig. 2. The *VHL* gene contains 3 exons, marked by empty boxes. Exon 1 contains a pentameric repeat in a 5' untranslated area of the gene (cross-hatched area). Exon 3 contains a large untranslated area (cross-hatched area). The upper numbers identify the nucleotide location of the open reading frame and intron breaks. Exon 1 contains a rare *Not I* enzyme cutting site (adapted from Latif et al. *[12]*).

9. CORRELATIONS OF GENOTYPE WITH PHENOTYPE

Once the *VHL* disease gene had been identified, correlations were made between germline *VHL* gene mutation and clinical manifestations. Clinical heterogeneity is a feature of VHL, with some families predominantly developing pheochromocytoma, renal cancer, or CNS hemangioblastoma. To evaluate this relationship, Chen et al. examined the findings in 114 VHL families *(57)*. Insertion, microdeletion, nonsense mutation, deletion, or missense mutation was identified in 75% of the families (85 of 114). Mutations were detected in all three coding exons, with greater frequency in the 3' end of exon 1 and the 5' end of exon 3. Exon 2 had a small number of mutations.

Families with VHL disease were grouped clinically by the absence (VHL type I) or presence (VHL type II) of pheochromocytoma *(57)*. The type of *VHL* gene germline mutation in type I families was different from those found in type II families. Virtually all (96%) mutations in VHL type II kindreds (VHL pheochromocytoma families) were found to be missense mutations, which result in amino acid substitutions, compared to 44% of VHL type I families ($p = 0.00001$) *(57)*. In codon 238, 43% of VHL type II mutations occurred in the 5' end of exon 3 of the *VHL* gene. It was also observed that the majority of *VHL* gene mutations found in families with kidney cancer without pheochromocytoma had mutations which should severely alter the structure of the VHL protein. These mutations were predicted to either compromise stability of the mRNA message, lead to truncation mutations, or interfere with cellular localization of the protein.

10. *VHL* MUTATIONS IN SPORADIC CLEAR CELL RENAL CANCER

In order to determine the role of *VHL* gene mutations in the development of sporadic or nonhereditary clear cell renal cancer, Gnarra et al. evaluated renal tumors from 110 patients (Fig. 3B) *(32)*. Loss of one copy of the *VHL* gene was detected as LOH in 98% of clear cell renal tumors. VHL gene mutations, which inactivate the second allele, were found in 57% of tumors. Mutations were detected in each exon of the *VHL* gene, with a high percentage, 45%, occurring in exon 2. Both missense and deletion mutation were found, suggesting exon 2 contains an important function of the protein *(2,32)*. Similar findings have been reported by a number of investigators from diverse geographic populations *(58–62)*.

An alternative mechanism of inactivation, hypermethylation, has been described in 11 *(63)* to 19% *(64)* of sporadic clear cell renal carcinomas. Regions of CpG-rich DNA are often found in and around the 5' regulatory areas of genes, and methylation in these areas is thought to lead to transcriptional inactivation *(65)*. No tumor with hypermethylation studied by Herman et al. expressed the *VHL* gene by Northern blot analysis *(64)*.

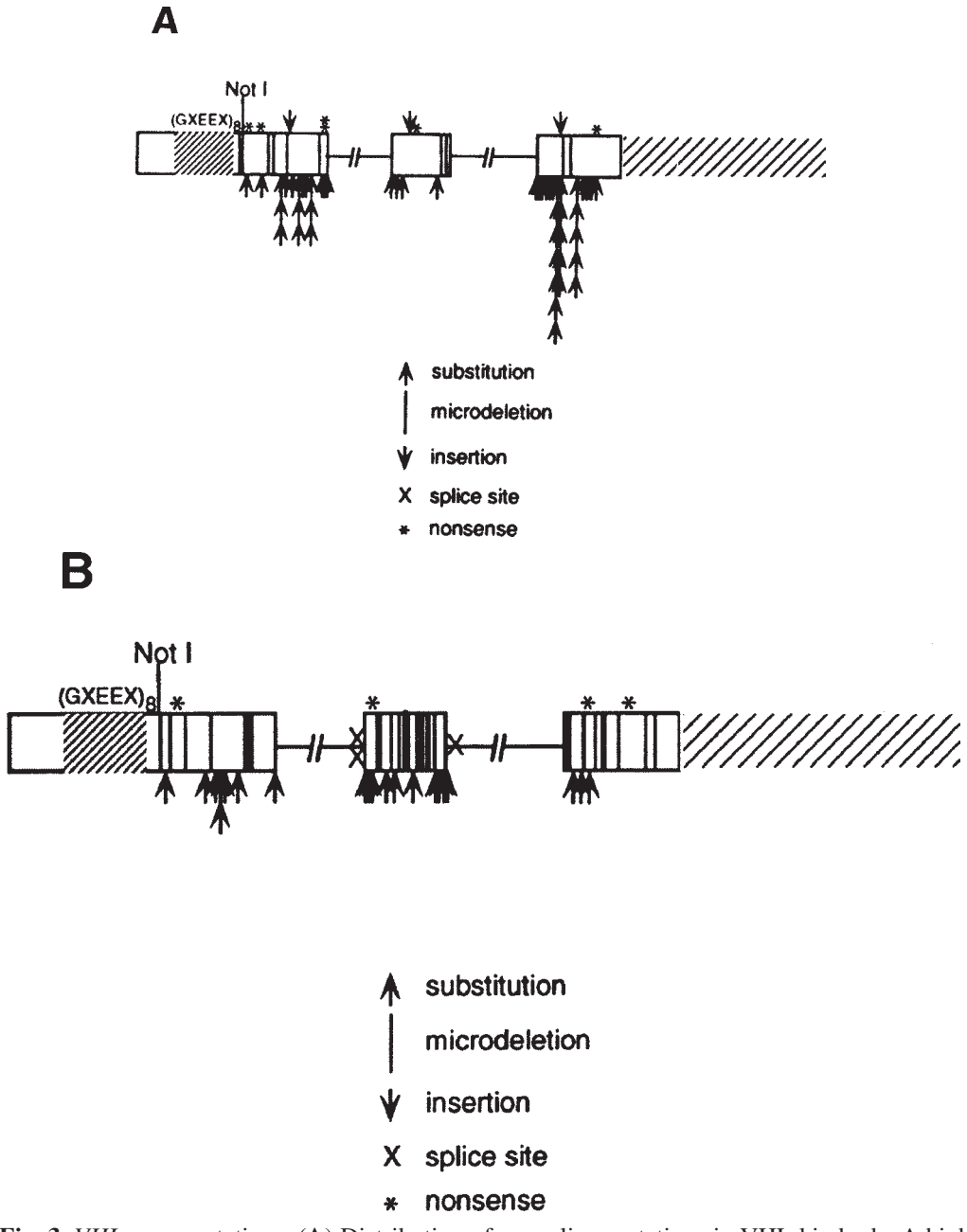

Fig. 3. *VHL* gene mutations. (**A**) Distribution of germ-line mutations in VHL kindreds. A high frequency of mutations have been clustered at the 3' end of exon 1 and the 5' end of exon 3. Mutations in exon 2 have been observed with less frequency (from Chen et al. *[57]*). (**B**) *VHL* gene mutations in noninherited or sporadic clear cell renal cancer. The mutations are clustered between the 3' end of exon 1 and the 5' end of exon 3, with many mutations found in exon 2 (from Gnarra et al. *[32]*).

These findings of a very high degree of *VHL* gene LOH, with at least 53 to 76% inactivation of the second allele, support the role of the *VHL* gene as a tumor suppressor gene important in clear cell renal cancer. In addition, the detection of *VHL* gene mutations in

small, clinically localized sporadic renal cell cancers (<2 cm) suggests that inactivation of this gene is an early event in tumor development *(66)*.

Sporadic nonrenal tumors have also been evaluated for *VHL* mutations. Gnarra et al. evaluated 119 tumors from 11 different tumor types, including lung, breast, and ovarian lung cancers *(32)*. No *VHL* gene mutations were detected in any of these tumors.

11. OTHER GENETIC ABNORMALITIES IN SPORADIC CLEAR CELL RENAL CANCER

Duplication of the chromosome 5q22-qter segment has been identified in 27 to 75% of clear cell renal cancers *(67–72)*. Kovacs proposed that mutation of a gene distal to the Adenomatous Polyposis Coli (*APC*) and Mutated in Colorectal Cancer (*MCC*) genes on chromosome 5q21 was associated with progression of clear cell RCC *(71)*.

The *p53* tumor suppressor gene is located on chromosome 17p13. *p53* is the most commonly mutated gene in human cancer and is thought to control cell cycling between the G1 and S-phase *(73,74)*. Loss of heterozygosity on chromosome 17p has been reported in 0 to 22% of clear cell RCC, most frequently in patients with advanced tumors *(35, 75,76)*. Reiter et al. performed RFLP analysis of 29 cell lines derived from patients with metastatic disease *(77)*. Fourteen (48%) evaluable cell lines showed LOH in the area of the *p53* gene. Furthermore, *p53* gene mutations were detected in 11 of 33 cell lines (33%). Oda et al. studied carcinomatous and sarcomatous portions of 14 sarcomatoid RCC by polymerase chain reaction (PCR), subcloning, and sequencing the *p53* gene *(78)*. Sarcomatoid portions showed a 78% mutation rate for the *p53* gene, compared to a 14% mutation rate found in the carcinomatous portions. These findings suggest abnormalities of *p53* are common, and might be related to tumor progression.

Wu et al. examined chromosome 14q, and found DNA loss in 36.7% of tumors *(79)*. These deletions were significantly correlated with higher stage ($p = 0.01$), histologic grade ($p = 0.01$), and worse-patient outcome ($p < 0.001$). LOH has also been identified on chromosomes 8p and 9p to correlate with a higher nuclear grade and advanced tumor stage. These promising prognostic markers will require larger studies to corroborate these findings.

Characterization of HCRC, as well as the hereditary form of clear cell RCC found in *VHL*, supports inactivation of the *VHL* gene as an initial event leading to clear cell RCC *(12)*. The development of sporadic clear cell RCC, which appears identical to the inherited form, is thought to have a similar origin. The frequent detection of *VHL* gene mutations, which would inactivate both copies of the *VHL* gene, support its role as a tumor suppressor gene *(32,63,64,80,81)*. The detection of these mutations in localized and advanced clear cell renal cancers suggests it has a critical role in the origin of this tumor *(12,32)*. Additional genetic changes are thought to contribute malignant growth and expansion of the tumor and contribute to progression of the disease.

12. PAPILLARY RENAL CELL CARCINOMA

Papillary renal cancer (PRC) is characterized by papillary or tubulopapillary architecture, and accounts for 10–15% of renal tumors *(41,82–84)*. PRC is morphologically and cytogenetically distinct from clear cell and chromophobe renal cancers *(41,82,83,85)*. The clinical behavior of these tumors may also be different. Survival rates of patients

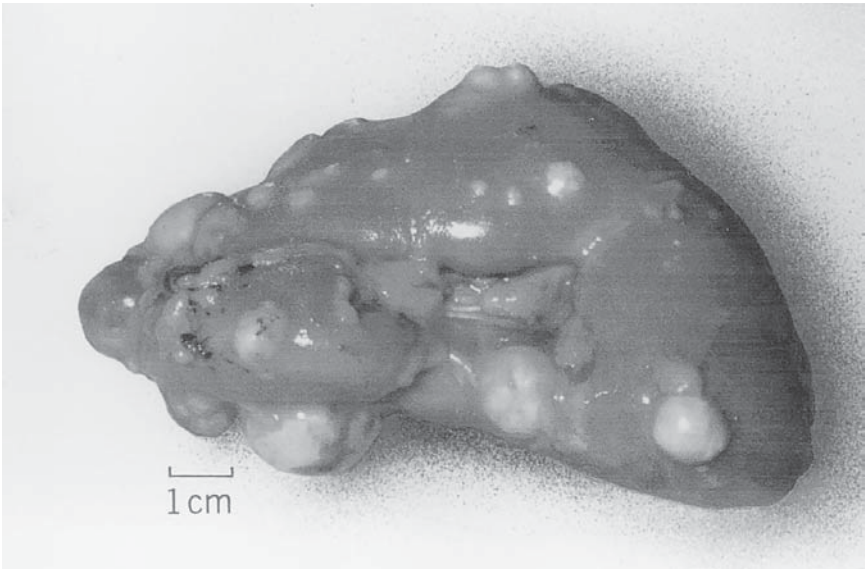

Fig. 4. Patients with hereditary papillary renal cancer are characterized clinically by the development of multiple, bilateral solid renal tumors, with basophilic papillary histology. In contrast to VHL patients, renal cysts are not encountered (from Zbar et al. *[16]*).

with similar stage or grade tumors have been reported to be higher in patients with PRC, compared to patients with clear cell renal cancer *(84,86)*. Males are more often affected with PRC than in clear cell renal cancer *(82,87)*. PRC (predominantly the eosinophil variant) occurs more frequently in patients with end-stage renal disease than in the general population *(88,89)*. Among the papillary histologic tumor types, basophil PRC appears to occur twice as frequently as eosinophil PRC *(85)*.

12.1 HPRC

PRC has been described in both a sporadic (nonhereditary) and hereditary form. Zbar et al. reported a hereditary form of basophilic PRC (HPRC) *(16,17,90)*, characterized clinically by bilateral, multifocal renal tumors without renal cysts (Fig. 4). Linkage analysis performed in HPRC families using probes along chromosome 7q localized a potential site for the *HPRC* gene to a 20 centimorgan interval at 7q31.3 *(18)*. The independent observation that trisomy of chromosome 7 is one of the most common cytogenetic findings in both sporadic and HPRC suggested this location contained a gene important in the development of basophilic PRC. Cytogenetic analysis of renal tumors enucleated from a patient with HPRC localized an area of duplication to the distal portion of chromosome 7q21–35 *(18)*.

The *HPRC* gene was recently identified as the protooncogene *met*, located on chromosome 7q31.1–34 *(18)*. Activating missense mutations were identified in the tyrosine kinase domain of the *met* gene in the germline of affected family members *(18)*. Trisomy-7 and nonrandom duplication of the mutant *met* allele were found in HPRC renal carcinomas (Fig. 5) *(91)*. A subset of histologically similar sporadic PRC were also found to have mutations of the *met* gene *(18)*. These findings supported the action of *met* as an oncogene, in contrast to the *VHL* tumor suppressor gene.

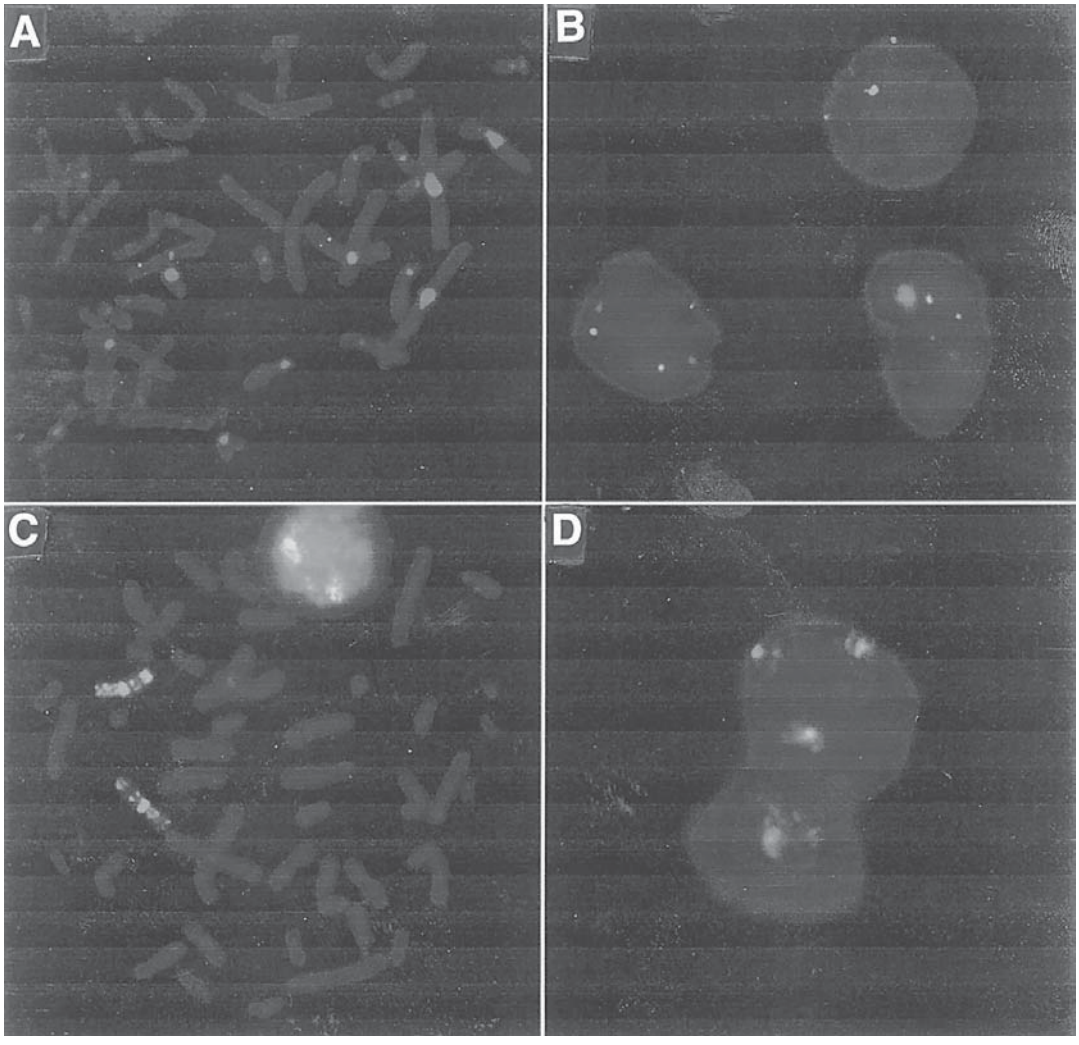

Fig. 5. Fluorescent In-Situ Hybridization (FISH) analysis of peripheral blood lymphocyte cell line and renal tumors from HPRC patients. (**A**) FISH of metaphase chromosomes derived from peripheral blood cultures. A cosmid clone containing the *met* gene (red signal) and a satellite centromeric probe (green signal) specific for chromosome 7 are shown. Two copies of the *met* gene (red signal) are seen. (**B**) FISH on renal tumor touch-preparation showing 3 copies of *met* gene (red signal) and a centromeric satellite probe located on chromosome 17 (green signal). (**C**) FISH of peripheral blood cell line from the patient in (**A**), using a chromosome 7-specific painting probe. Two germline copies are seen. (**D**). FISH on renal tumor touch-preparation from patient in (**B**). Three copies of chromosome 7 are seen in the tumor cells using a chromosome 7-specific painting probe (red signal) and a chromosome 7 satellite probe (green signal) (from Zhuang et al. *[91]*).

13. RENAL ONCOCYTOMA

13.1. Hereditary Renal Oncocytoma

Renal oncocytoma accounts for 3–5% of renal tumors and is thought to originate from the intercalated cells of the distal renal tubule and collecting duct *(10,92)*. Histologically, this tumor has features that overlap with other renal neoplasms with a preponderance of

eosinophilic (granular) cytoplasm, including eosinophilic PRC, chromophobe RCC, and the granular cells found in some forms of clear cell renal cancer *(94)*. Weirich et al. reported five families in which multiple members of the kindreds were affected with oncocytoma, an apparent familial form of oncocytoma *(19)*. These patient's tumors were multiple and bilateral. No metastatic disease was observed, similar to sporadic, or noninherited, renal oncocytoma. These findings suggest there is a genetic predisposition to develop renal oncocytoma, similar to other forms of familial renal tumors.

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6 Screening for Renal Cell Carcinoma

Evan B. Cohn and Steven C. Campbell

Contents

1. INTRODUCTION

Interest in screening for urologic malignancies has increased in recent years primarily because of the advent of the clinical use of prostate-specific antigen. Although the debate regarding an actual survival benefit from earlier and more frequent detection has not been settled in the case of prostate cancer, it is clear that early detection in the case of renal cell carcinoma (RCC) would be beneficial. In this chapter, we will assess the rationale for screening for RCC, examine the diagnostic tests available for screening, and review screening recommendations for target populations, including the recently described familial forms of RCC.

2. SCREENING THE GENERAL POPULATION

2.1. Paradigms and Rationale

In 1998, there were 29,900 new cases of RCC diagnosed in the United States; 17,600 in males and 12,300 in females, representing approximately 3% of all noncutaneous malignancies. Deaths caused by metastatic disease numbered 11,600, reflecting the significantly higher mortality rate associated with RCC when compared to the other common urologic malignancies *(1)*. Although the most recent data document 5-yr cancer-specific survivals of 60% for newly diagnosed patients, which is a significant improvement over outcomes for patients diagnosed during the 1970s and 1980s, it is clear that RCC remains a lethal malignancy *(1)*.

A number of factors make screening for this disease pertinent. Most importantly, RCC is generally only curable when localized and amenable to complete surgical resection. Secondary to the sequestered location of the kidneys within the retroperitoneum, RCC

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often remains asymptomatic until locally advanced or metastatic disease has developed. Despite improved understanding of the molecular basis of RCC and active research into the treatment of advanced disease, our ability to salvage patients with advanced disease remains limited. For these reasons, RCC remains primarily a surgical disease necessitating early diagnosis in order to maximize the opportunity for cure.

Several studies have demonstrated an advantage to early or incidental diagnosis of RCC *(2–7)*. Konnack and Grossman found that 86% of incidentally discovered tumors were Robson's stage I or II, compared with only 46% of symptomatic tumors *(6)*. Their findings were echoed by Thompson and Peek, who reported that 87% of incidentally discovered tumors were localized, compared with 42% for symptomatic tumors *(2)*. In both series, 5-yr survival rates were significantly improved for patients with incidentally discovered RCC. More recent data from Rodriguez-Rubio and colleagues also confirm a strong association between incidental detection, localized tumor stage, and improved survival *(3)*. The potential benefits of early detection of RCC are clear and with the ready availability and accuracy of noninvasive imaging modalities interest is screening for RCC has grown.

2.2. Problems with Generalized Screening

The primary factor that limits the widespread implementation of screening for RCC is the relatively low incidence of RCC in the general population (8.9 cases/100,000/yr) *(8)*. A screening test would need to be almost 100% specific to avoid an unacceptably high false-positive rate. For example, even if a screening test were 99% specific, 1000 people out of a population of 100,000 would undergo unnecessary, expensive, and potentially harmful diagnostic or therapeutic procedures to diagnose a small number of malignancies. Additionally, even if the tests were 100% sensitive and specific, the incidence of RCC is so low that the test would not be considered cost effective in today's cost-conscious health care environment. Even when one considers populations with established risk factors for RCC, such as male sex, increased age, and heavy tobacco use, generalized screening would be difficult to justify, as the increase in relative risk associated with each of these factors is, at best, two–threefold *(9,10)*. Another confounding factor is the occurrence of clinically insignificant tumors, such as renal adenomas, which are found at autopsy in 10–20% of individuals, and other benign or slow growing tumors *(11–13)*. There is clearly a possibility that such clinically insignificant lesions could be detected, leading to unnecessary evaluation and treatment. All of these factors would mitigate against generalized screening efforts for the detection of RCC.

3. SCREENING METHODS

3.1. Urinalysis

Urinalysis has traditionally been performed as part of a routine annual checkup by many primary care physicians and occasionally leads to an early diagnosis of RCC because of associated microhematuria. This will soon become an uncommon occurrence as a number of reports have recently been published in the primary care literature demonstrating a relatively low yield for routine urinalysis during health care maintenance *(14,15)*. It is thus likely that many health care plans will elect not to cover this simple and inexpensive test as a screening modality.

Several studies have examined the benefits of routine screening for asymptomatic microhematuria (AMH). In general, the results have been mixed and seem to hinge on

a number of factors including the definition of microhematuria, the demographics of the study population, and the extent of urologic evaluation *(16)*. Only a few population-based studies have been performed, and in many, an adequate urologic evaluation of patients with microhematuria was not consistently pursued *(17–25)*. For instance, in the study by Mohr and colleagues, a high proportion of patients with AMH were not referred for urologic evaluation *(24)*.

Overall, the incidence of AMH in the general population was in a range of 3–13%, and the diagnostic yield in such patients has been relatively low, especially regarding RCC *(19,20,22,24,25)*. In series that have incorporated both cystoscopy and upper tract imaging for routine evaluation, approximately 20% of patients with AMH have been found to have significant urologic disease, most commonly, renal calculi or transitional cell carcinoma, and a diagnosis of RCC has been an uncommon event *(17–23)*. For instance, in the study by Thompson, which screened 2005 military men older than 40 with urinalysis, the incidence of AMH was 4%, and despite a complete urologic evaluation of this subgroup, only 19 patients or 0.94% were found to have "significant urologic disease." However, the number of patients diagnosed with bladder cancer ($n = 1$) or RCC ($n = 0$) was exceedingly low *(25)*. This is not a fault of the screening methodology or the diligence of evaluation; given the low incidence of RCC, it is statistically likely that none of the screened subjects actually had RCC. Other studies also suggest a low yield of RCC in patients evaluated for AMH.

Currently, there is no compelling evidence that screening the general population for AMH would significantly impact upon the outcomes or natural history of RCC. In part, this may be caused by the fact that early-stage RCC is not invariably associated with microhematuria, as it is a parenchymal, rather than a urothelial, based malignancy, and when hematuria does occur, it is often intermittent. In the screening study by Tosaka and colleagues, which incorporated ultrasonography and urinalysis, less than half of the 35 patients with RCC had hematuria, either gross or occult, and those with hematuria had either gross hematuria or other tumor-related symptoms. Strikingly, none of the patients in this series had AMH *(26)*. Other series in the literature also suggest that it is uncommon for RCC to present with isolated microhematuria, which implies that screening for RCC with urinalysis may be inherently flawed *(9)*. In addition, Messing and colleagues have shown that microhematuria can be intermittent in patients with urologic cancers, making urinalysis an even less precise test *(27)*.

3.2. Ultrasound

There have been two major efforts in the literature examining ultrasound as a screening modality in an asymptomatic population. The first group of studies were conducted in Japan and evaluated the screening of 45,905 patients *(26,28,29)*. Overall, 469 lesions requiring further evaluation were identified. Follow-up CT or angiography revealed a total of 35 patients with RCC, three with upper-tract transitional cell carcinoma, and 16 with renal calculi. This represents an incidence of RCC of 77/100,000, which was more than 10-fold higher than expected for the general population. Overall, 19 of the patients with RCC were asymptomatic and all were found to have tumor confined to the kidney. Cancer-free survival was significantly improved in this group of patients when compared to symptomatic patients. This led the Tosaka group to conclude that renal ultrasound was a "safe, innocuous, and inexpensive" tool for the detection of localized RCC, and they promoted its use as a screening tool *(26)*. A similar study was conducted in Canada where

1000 asymptomatic executives underwent abdominal ultrasonography *(30)*. Of this group, four were found to have RCC, and in the follow-up study where 7925 patients were screened, an additional 23 RCCs discovered. The overall rate of detection of RCC was approximately 300/100,00, much higher than anticipated, even when accounting for the male predominance and advanced age of the study population. Again, there was a predominance of organ confined RCC in the screened populations.

Two abstracts recently presented at the annual meeting of the American Urological Association have also argued in favor of ultrasonography for screening for RCC *(31,32)*. Koeneman et al. reported a yield of 16 RCCs from a population of 5898 elderly patients (age range 50–79), which would represent a significantly increased incidence of 271/100,000 *(32)*. All but one tumor was stage T2 or greater, suggesting that incidental detection of clinically insignificant tumors was not occurring. With limited follow-up, the majority of patients were free of disease after nephrectomy. The authors argued that targeting at risk populations, such as older, primarily male patients, as well as scanning for other potentially life-threatening conditions, such as aortic aneurysms, can increase the yield of the study, and thereby improve the cost effectiveness of the screening efforts. They also employed a technician rather than a radiologist to perform the initial ultrasonographic evaluation in an effort to reduce the associated costs. In Germany, retroperitoneal ultrasonography is routinely performed by many general practitioners or urologists which significantly reduces costs and enhances its appeal for generalized screening endeavors *(31)*.

There are problems with extrapolating data from the previously mentioned studies in favor of generalized screening. Again, even with the higher-than-expected yield of RCC in the various studies, the cost of such an undertaking would be prohibitive in the managed care era. Furthermore, in many of these studies there is no way to determine the false-positive rate. False positives would be those lesions that were discovered and treated that would not have been biologically threatening. It is important to keep in mind that although several studies have documented an improved outcome for incidentally discovered RCC, there has been no randomized, prospective study demonstrating a benefit from an active screening program for RCC. Parallels between ultrasound and PSA can be drawn in that they are both effective at finding tumors, the detected tumors tend to be smaller and more often organ confined, and with the limited data available, the outcomes of treated patients appear to be improved. However, in both cases a beneficial effect on overall survival for the population at large has not been established, leaving the topic open to debate. The same issues of lead and length-time bias that have dominated the debate about prostate cancer can be invoked in the evaluation of screening for RCC.

As ultrasonographic technology continues to evolve, it may eventually become a more appropriate tool for screening. If the transducer were to become as user friendly and commonplace as a stethoscope it is conceivable that it could be incorporated into the routine physical examination of older, at-risk patients. This, more than anything, would permit general screening of the retroperitoneal organs to become a more reasonable and cost-effective proposition.

3.3. Molecular Genetics

The recent discoveries pertaining to the molecular and genetic defects that accompany RCC have been staggering *(33–39)*. The pathogenic role of von Hippel-Lindau (VHL) tumor suppressor gene mutations in both the familial and sporadic forms of the common clear cell variant of RCC has now been defined, the specific defects that are present

Table 1
Familial RCC Syndromes

Syndrome	*Molecular Abnormality*	*Clinical Manifestations*
von Hippel-Lindau	Inactivation of the VHL tumor suppressor gene (3p25-26)	RCC, retinal angiomas, pheochromocytoma, and cerebellar, medullary, or spinal hemangioblastomas
Hereditary Papillary RCC	Mutations activating the MET protooncogene (7q31-34)	Multifocal papillary RCC's
Non-VHL Clear Cell RCC	Undefined in most instances	Multifocal RCC
Familial Oncocytoma	Undefined	Multifocal oncocytomas

in the hereditary form of papillary renal cell cancer have come to light, and a familial form of renal oncocytomas has recently been reported *(33,35–37,40–42)*. The complete sequence of the wild-type *VHL* gene, which is located on chromosome 3p25–26, has been determined, and advanced molecular techniques, such as polymerase chain reaction (PCR), analysis for single-strand conformational polymorphism, and DNA sequencing, are now readily available and can be used to define the genetic alterations underlying the development of RCC in specific individuals or families with the VHL syndrome *(34,43)*. Mutation or inactivation of this gene by DNA methylation have been found in almost all families with the VHL syndrome and in 60–75% of sporadic clear cell RCCs *(34,45)*. Even with this wealth of information, application of molecular screening to the population at large remains impractical. The cost of such an endeavor would be prohibitive given the relatively low incidence of RCC in the general population, as well as the expense of such an undertaking using today's technology. In addition, molecular diagnosis of patients at risk for sporadic tumors would require renal tissue, as the associated mutations tend to be isolated to the organ of interest, rather than generally distributed as is the case for the less common familial forms of the disease. Also, for a significant proportion of sporadic clear cell tumors, as well as other histologic subtypes, the specific genetic alterations responsible for neoplastic transformation have not yet been elucidated *(45)*. At present, molecular screening is only likely to benefit patients with clinical findings or family history suggestive of a inherited germline mutation, which would include the various familial syndromes (Table 1), which will be discussed in greater detail in the following sections. Information about molecular screening for the familial forms of RCC can be obtained on the internet at www.ncifcrf.gov/kidney or by directly contacting the Urologic Oncology Branch of The National Cancer Institute.

4. SCREENING POPULATIONS AT RISK

Although screening the entire population for RCC appears to be impractical, there are groups in whom the incidence of RCC is sufficiently high that screening may be beneficial. These groups include patients with end-stage renal disease (ESRD) and acquired renal cystic disease (ARCD), VHL disease patients and their families, and patients with other familial syndromes such as hereditary papillary renal cancer (HPRCC), tuberous sclerosis *(TS)*, and autosomal dominant polycystic kidney disease (ADPKD).

4.1. End-Stage Renal Disease and Acquired Renal Cystic Disease

In the United States, there are currently 260,000 patients with ESRD, with 195,000 receiving maintenance dialysis therapy *(46)*. Appropriate screening for RCC in this large patient population has been highly controversial, but appears to be warranted in certain instances.

The first series that reported the development of ARCD and subsequently solid tumors in patients receiving long-term hemodialysis was published in 1977 by Dunnill and colleagues *(47)*. ARCD, which has been defined as the presence of multiple cysts occupying at least 25% of the renal mass, appears to be the main risk factor for the development of RCC in patients with ESRD *(48–50)*. The onset of ARCD is variable and depends on the type and duration of renal replacement therapy. Hemodialysis is most commonly associated with ARCD: 30–45% of patients will demonstrate multiple cysts after 3 yr of dialysis and 80–95% will eventually qualify for the diagnosis of ARCD *(49–56)*. Overall, the main risk factor for developing ARCD appears to be the length of time on dialysis and not chronological age; therefore, pediatric patients can also be at risk *(53)*. Patients on peritoneal dialysis appear to have a lesser risk (30–50%) of developing ARCD and the condition has been noted to regress somewhat after renal transplantation *(49,57–59)*. It is also important to note that males develop ARCD at twice the rate of females, and males have a sevenfold-higher risk of developing RCC in the setting of ARCD *(54,55,60–62)*.

Several studies have confirmed the association between the development of solid renal tumors and ARCD. In Dunnill's original autopsy series of 30 patients on long-term hemodialysis, 14 (47%) were found to have ARCD, and all 6 patients with solid renal tumors had ARCD *(47)*. Ishikawa diagnosed three RCCs on initial radiographic screening of 96 patients on chronic hemodialysis, and three more were diagnosed when this group was followed longitudinally with serial abdominal CT scanning *(55,58)*. Again, all six patients with RCC had ARCD. Review of the literature demonstrates that about 80% of hemodialysis patients with RCC have had ARCD. There also appears to be an increased incidence of renal adenomas in patients with ARCD. Overall, about 20–40% of patients with ARCD have been found to have solid masses on pathologic study, which is twice the rate of renal adenomas found in the general population *(47,61,63)*.

Previous data suggesting that the risk of developing RCC are less in patients on peritoneal dialysis or after renal transplantation has now been challenged. In a recent series 5 of 129 renal transplant patients were diagnosed with RCC in the native kidneys, which would represent a markedly increased incidence of neoplastic transformation *(64)*. All tumors were confined to the kidney and the authors argued in favor of screening the native kidneys of asymptomatic transplant patients. Further data will be required to resolve this important issue.

There is active debate regarding the pathogenesis of ARCD and RCC in ESRD *(55,65)*. Theories range from an obstructive etiology caused by oxalate deposition to the accumulation of nondialyzable compensatory growth factors that in the presence of uremia may stimulate cyst formation and tumorigenesis. Impaired immune surveillance associated with uremia may also play a role. Recent studies suggest that the histologic subtypes and genetic defects found in tumors developing in the setting of ESRD and ARCD may differ from those observed in patients with sporadic RCC *(66–68)*. In the series by Hughson and colleagues, only 3 of the 17 RCCs associated with ESRD were classified as clear cell carcinomas, and only one demonstrated the classic chromosome 3p deletions associated with this histologic subtype *(67)*. A subsequent study using more sophisticated molecular

techniques reported that 6 of 18 RCC's associated with ESRD demonstrated chromosome 3p alterations, which is still much less than would be expected for sporadic RCC *(68)*. The pathogenic factors accounting for the unique distribution of histologic subtypes and genetic alterations found in RCC associated with ESRD have not been defined.

Review of the various series in the literature suggests that approximately 1–2% of patients with ARCD will develop RCC, and the increased risk of RCC in this population has been estimated to be anywhere between 5- and 100-fold higher than the general population *(49,51,53–55,69)*. Approximately 15% of patients with RCC in the setting of ARCD have metastatic disease at diagnosis, demonstrating the potential for aggressive behavior in these tumors *(53,55,70–72)*.

The increased incidence of ARCD and RCC in patients with ESRD has led most authors to recommend yearly screening with ultrasonography until cysts are identified, and subsequently with CT scanning *(48,50,54,61,72–74)*. CT is regarded as the superior imaging modality in this population, but both studies can be difficult to interpret secondary to ARCD. To help control the cost of screening, which has been a major concern, many authors have recommended that screening be delayed until the third year of dialysis, or that it could be performed less frequently in women and those on peritoneal dialysis.

The utility of universal screening in patients with ESRD is controversial and has been challenged in the literature *(51,75,76)*. The life expectancy of patients on dialysis is short with 5-yr and 10-yr survival of 28% and 10%, respectively *(77)*. Most will succumb to other medical diseases rather than RCC and some have argued that screening is unlikely to significantly impact upon overall survival *(78)*. Even when active screening is being employed, metastatic RCC can develop, in part because of the inherent difficulties with diagnosing small tumors amidst the altered intrarenal architecture associated with ARCD *(51)*. There may also be an increased risk of diagnosing clinically insignificant tumors in light of the increased incidence of adenomas in this population. All of these factors could mitigate against the benefits of screening.

A reasonable compromise would be to target subsets of dialysis patients that are relatively young and without major comorbidities, as they are most likely to benefit from screening (Table 2). An estimate of the relative risk of developing RCC based on sex and duration and type of renal replacement therapy should also be taken into account. As always, the decision about screening should be made on an individual basis, respecting the wishes of the well-informed patient.

4.2. VHL Patients and Families

VHL disease is a multisystem neoplastic disorder with nearly complete penetrance by age 60 *(43,79–82)*. In the majority of patients, the disease is transmitted in a familial, autosomal dominant manner; less than 10% of cases are thought to be caused by spontaneous mutation *(43,79)*. Afflicted individuals have a predisposition toward developing highly vascular tumors of neuroectodermal origin. The six major clinical manifestations are: retinal angiomatosis, RCC, pheochromocytoma, and cerebellar, medullary, or spinal cord hemangioblastomas *(43,79,80,82–86)*. A diagnosis of VHL has traditionally required a minimum of two of these major manifestations, or one in the context of a positive family history, but a more exact diagnosis of symptomatic or asymptomatic individuals is now possible using molecular analysis *(82)*. Important clinical clues to the diagnosis of VHL include early onset or multifocal RCC, a family history of blindness, renal malignancy, or CNS tumors or paralysis, or the presence of multiple renal or pancreatic cysts, epididymal

Table 2
Screening Target Populations for Renal Cell Carcinoma

- Patients with end-stage renal failure:
  - Consider screening only relatively healthy patients with a long life expectancy
  - Screen initially with ultrasound during the third year on dialysis. When cysts develop, obtain CT periodically
- Patients with known von Hippel-Lindau disease:
  - Obtain CT or ultrasound biannually beginning at age 20
  - Periodic clinical and radiographic screening for nonrenal manifestations
- Relatives of patients with von Hippel-Lindau disease:
  - Obtain genetic analysis
    - Patients with a positive genetic analysis: Follow screening recommendations outlined for patients with known VHL
    - Patients with a negative genetic analysis: Consider abdominal ultrasound as a screen for major renal or adrenal manifestations of disease. Less stringent follow-up required
- Relatives of patients with other forms of inherited RCC, i.e., hereditary papillary renal cell carcinoma or familial oncocytoma:
  - Screen periodically with ultrasound or CT and consider molecular analysis
- Patients with tuberous sclerosis:
  - Consider periodic screening with CT or ultrasound
- Patients with autosomal dominant polycystic kidney disease:
  - Routine screening is not justified

cystadenomas, or inner-ear tumors, which have recently been described in association with the VHL syndrome *(43,79,82)*. As RCC is one of the more common manifestations of VHL, it is important for urologists to be familiar with the various manifestations and to be knowledgeable about the evaluation of VHL patients and their families *(82)*.

Many of the nonrenal tumors associated with VHL can lead to significant morbidity or mortality, which can be prevented if identified during the asymptomatic phase. Retinal angiomatosis, which is often the earliest manifestation of the syndrome, occurs in 50–60% of VHL patients, and can lead to retinal detachment and blindness *(43,83)*. Central nervous tumors, which tend to develop in the third decade of life, are found in 40–50% of patients, and can lead to quadriplegia. Until recently, they represented the most common cause of death in VHL *(43,85)*. Early diagnosis of the retinal or CNS manifestation is particularly important, as several studies have confirmed that skilled laser surgery or neurosurgery, when delivered in a timely fashion, can prevent many of these catastrophic outcomes *(79,82,84,87)*. Pheochromocytoma tends be clustered in certain families, so its incidence is quite variable, affecting anywhere from 0–50% of patients in a given family. Identification of pheochromocytoma is important because it places the patient at risk for hypertensive crises, particularly if unrecognized at the time of surgery.

The malignant potential of RCC in VHL remains somewhat controversial, as does the management of this subgroup of patients. Several reports suggest that RCC in VHL may have less malignant potential, but this is difficult to reconcile with data demonstrating that RCC is now the most common cause of death in this syndrome *(79,81,84)*. In one large series, metastatic RCC accounted for 42% of the deaths, so it is clear that this entity

must be treated with great respect *(84)*. In general, nephron sparing surgery has been preferred, but treatment must be individualized, taking into account the extent and grade of disease, general medical condition, and the presence of intercurrent sequellae of VHL *(88)*. Recent data demonstrate a high local-recurrence rate during long-term follow-up after partial nephrectomy, which is in concordance with other studies showing an exhorbitant number of occult lesions in VHL kidneys *(89–91)*. Nephron-sparing surgery should thus be viewed primarily as a temporizing measure, and it must be combined with close follow-up, and the recognition that further surgery, often completion nephrectomy, may be required *(89)*. Bilateral nephrectomy with subsequent transplantation has also been described and may be the best option for patients with extensive bilateral disease, particularly if high grade *(88,89,92)*.

Like most tumor suppressor genes, loss of function of both of the alleles for VHL is required for tumors to develop. Patients with VHL are born with one mutant allele that has been transmitted from one of the parents in an autosomal-dominant fashion *(82)*. They then only require random mutation of the second allele in any of the affected organs to develop tumors, and this accounts for the early and multifocal presentation of RCC in VHL patients. In contrast, patients with sporadic RCC must acquire somatic mutations of both alleles within the same cell, and as each event occurs with relatively low frequency, sporadic RCC tends to be late onset and unifocal in the majority of instances *(45)*. The exact mechanism by which loss of function of the VHL protein leads to the development of RCC or other tumors has not been elucidated, but interactions with various transcription factors such as the Elongins and CUL-2 may play a role *(82,93–95)*. Deregulation of the expression of vascular endothelial growth factor appears to contribute to the increased vascularity of VHL-related tumors, and more recent reports suggest that the wild-type VHL protein may regulate cellular differentiation and the expression of fundamentally important extracellular matrix proteins *(39,96–100)*.

The molecular genetics of VHL are now well defined (*see* Chapter 5), allowing for molecular screening, which represents a major step forward for these patients and their families *(34,35,101–103)*. Through a combination of PCR amplification, analysis for single-strand polymorphism, and DNA sequencing, the particular molecular alteration causing the disease in any given family can be identified, and asymptomatic individuals can be screened early in life to determine their genotype with respect to this locus *(34,36,37)*. This represents a significant advance from previous techniques, which tested for loss of heterozygosity (LOH) at closely linked markers. This form of analysis, which was reported in a landmark paper by Glenn and colleagues in 1992, proved to be highly accurate (no false positives and only one false negative when evaluating a population that included 9 with and 33 without the disease) but labor intensive, and it failed to yield diagnostic information in 15% of families, as some were found to be homozygous at all of tested loci *(34,36,37,104)*.

A correlation between genotype and certain phenotypic manifestations of VHL has also recently been described and should prove to be clinically useful. Alterations at codon 238, which are missense mutations leading to amino acid substitutions, appear to be particularly important, as they identify families at risk for pheochromocytoma *(37,82)*. In contrast, families that do not develop pheochromocytoma have been found to possess mutations that more severely impact upon the VHL protein structure, often leading to a trunctated protein. A genetic classification of VHL mutations with correlation to phenotypic expression is currently evolving.

Molecular screening should be strongly considered for all patients suspected of having VHL and for close relatives of patients known to have the disease (Table 2). As discussed earlier, identification of patients with specific *VHL* gene defects can direct clinical and radiographic screening efforts, and those individuals found to be wild type for both alleles can be spared life-long screening studies, which can be extremely stressful and expensive. A list of centers that perform mutation analysis for VHL is provided in the recent review by Neumann et al. *(36)*.

For those patients found to have VHL by molecular techniques or clinical presentation or those in whom a determination cannot be made, routine radiologic screening is mandatory. This screening includes: (1) a yearly physical exam and blood pressure measurement; (2) annual ophthalmologic exam and fluourescein angiography beginning at age 6; (3) brain and spinal MRI every three years beginning at age 15–20; (4) measurement of serum or urinary catecholamines at age 15-20, then repeat if hypertension develops; and (5) abdominal CT scan at age 20, then CT or renal ultrasonography biannually *(105)*. Audiometric evaluation should also be considered given the association with inner ear tumors *(82)*. Utilization of this type of screening allows diagnosis of clinically significant lesions while still asymptomatic and amenable to therapy.

4.3. Hereditary Papillary RCC

Papillary renal cell cancer is the second most common histologic subtype of RCC, accounting for about 10% of all malignant renal tumors. These tumors are characterized by distinct abnormalities of chromosomes 7 and 17 rather than 3p, an increased incidence of multifocality, decreased vascularity when compared to the more common clear cell variant, and, although admittedly still somewhat controversial, a slightly increased propensity for low-grade and organ-confined status *(106–111)*.

Recent data from researchers at the National Cancer Institute, led by Linehan and Zbar, have defined the clinical and molecular features of a new familial syndrome of RCC, hereditary papillary RCC (HPRCC) (Table 1) *(41)*. In their report, the authors described 10 families with HPRCC, with 41 affected members, 29 male and 12 female. A strong case was made for familial transmission: in addition to the clustering of cases within multiple generations of certain families, there was concordance between identical twins, and early age of onset (mean of 45 yr) and bilateral, multifocal disease was observed in the majority of cases. High-grade disease (grades 3 or 4) was found in a significant proportion of patients (>50%) and the mean survival of affected individuals was only 52 yr of age. The number of deaths caused by RCC was not defined, but it is clear that this represents a disorder with considerable malignant potential. In three instances, asymptomatic family members were identified through screening abdominal ultrasonography. Interestingly, as in the case of RCC in the VHL syndrome, penetrance was far from complete, suggesting a role for epigenetic factors *(41)*. A number of additional families with HPRCC have subsequently been identified and characterized by the group at the NCI, as well as other investigators *(112,113)*.

The molecular basis for the development of HPRCC (*see* Chapter 5) has now been elucidated with the recent description of activating mutations of the *MET* protooncogene, which segregate with affected family members *(113–115)*. The pattern of inheritance appears to be autosomal dominant. The protein product of this gene is a receptor tyrosine kinase expressed by epithelial cells, which transduces motility and cellular proliferation signals induced by the binding of hepatocyte growth factor, also known as scatter factor.

Schmidt and coworkers have shown that missense mutations lead to constituitive activation of the receptor, which predisposes to neoplastic transformation *(114)*. The gene is located on chromosome 7q31-34, which correlates well with the cytogenetics of sporadic papillary RCC, which often includes trisomy of 7 *(114)*. It has been hypothesized that this karyotypic abnormality could predispose to malignancy by increasing the molecular dosage of the *MET* protooncogene.

Molecular screening for HPRCC should be strongly considered in families suspected of having this syndrome. Until the accuracy of the molecular approach can be confirmed, standard radiologic techniques should also be used. The youngest patient with HPRCC in the NCI series was 18 yr old, which suggests that screening with ultrasonography or CT scanning should be initiated relatively early in life, but absolute recommendations for initiation and interval of screening are not yet available *(41)*.

4.4. Familial non-Papillary, non-VHL RCC

A number of familial aggregates of clear cell RCC, in the absence of other manifestations of VHL, have been reported in the literature *(116)*. In many of these cases, early age of onset and multifocal tumor have been reported, suggesting familial inheritance *(116)*. The distinction between familial clear cell RCC and VHL is not always clear and these patients should undergo thorough clinical and radiographic evaluation for the other manifestations of VHL, and molecular analysis for mutations of the VHL tumor suppressor gene should also be considered. Investigators at the National Cancer Institute would appreciate the opportunity to evaluate such families, as it could lead to the identification of additional genetic loci contributing to the development of RCC. In one such family, a balanced chromosomal translocation between chromosomes 3 and 8 was identified and shown to be coinherited along with RCC. Screening for this karyotypic abnormality led to the identification of three family members with asymptomatic RCC *(117)*. Two other families with predisposing chromosomal abnormalities have also been identified, but in most other instances, molecular analysis has not been informative *(116)*. Until more sophisticated molecular analysis becomes available, all members of families suspected of having familial non-VHL clear cell RCC should be evaluated with periodic renal ultrasonography or CT scanning (Table 2).

4.5. Familial Renal Oncocytoma

A familial form of renal oncocytoma has also been described (Table 1). Weirich and colleagues from the NCI recently described five families with a total of 15 patients with renal oncocytomas and presented a strong argument in favor of an inherited predisposition *(42)*. Concordance was observed between a pair of identical twins and multifocal, bilateral disease was common. However, mean age at diagnosis was 55.8 yr, considerably later than that observed for the other familial forms of RCC. Although some tumors demonstrated hemorrhage or cytoplasmic clearing, which are atypical features for renal oncocytomas, no patients developed metastatic disease and there were no tumor-related deaths. The natural history of this disorder and the role of screening have not been adequately defined.

4.6. Tuberous Sclerosis

There have been several reports of RCC in patients with tuberous sclerosis (TS), and although still somewhat controversial, the association appears to be more than coincidental

(118,119). The more common renal lesions are renal cysts and angiomyolipomas, and other major manifestations, which occur with variable penetrance, include epilepsy, mental retardation, and adenoma sebaceum, a distinctive skin lesion *(118,120)*. The hallmark lesion is the hamartoma of the cerebrum. Two gene loci responsible for this disorder have been identified and have been designated TSC1 and 2, with mutations of the latter gene predominating. As both TS and RCC are rare diseases, finding a strong association between them has been difficult and the literature remains contradictory. A number of studies have suggested an increased incidence of RCC in TS, and there is also a biologic basis for this association *(121)*. The Eker rat, which develops renal carcinoma at high frequency, has been found to harbor a germline mutation in the *tsc2* gene, which is homologous to the human *TSC2* gene. Replacement of the wild-type *tsc2* gene in transgenic rats blocks renal carcinogenesis, suggesting a causative role for *tsc2* mutations, and by analogy, perhaps also *TSC2* mutations in humans *(121)*. Furthermore, many RCCs in patients with TS have demonstrated early age of onset and multifocality, which suggests an inherited predisposition *(122)*. Such observations argue in favor of an increased risk of RCC in TS patients. However, a recent meta-analysis conducted by Tello et al. argues to the contrary *(123)*. This large series failed to demonstrate an increased incidence of RCC in patients with TS when compared to the general population.

It is clear that RCC in TS can pursue an aggressive course, with four of six patients in one series dying of metastatic disease. Most authors have recommended screening for renal lesions in this syndrome *(124–126)*. The impetus for screening in this population is actually twofold, as many patients also have angiomyolipomas that need to be followed and treated as clinically indicated *(127,128)*. Until resolution of the aforementioned controversies can be achieved, it would be prudent to screen patients with TS periodically with ultrasonography or abdominal CT scanning.

4.7. Autosomal Dominant Polycystic Kidney Disease

The literature pertaining to the relative risk of developing RCC for patients with ADPKD has been somewhat contradictory. Earlier reports suggesting an association between RCC and ADPKD have now been countered by larger series demonstrating that the risk of neoplastic transformation is not significantly increased above the general population *(129–132)*. In the series by Gregoir and associates, only 1 of 87 patients with ADPKD was found to have RCC, and recent autopsy studies suggest that although there may be more adenomas the incidence of RCC is not significantly elevated in this patient population *(130,131)*. The weight of evidence therefore suggests that routine screening of asymptomatic individuals with ADPKD would be associated with a relatively low yield, and is not without risk and expense. Both ultrasound and CT can be difficult to interpret in this patient population, and many patients would be exposed to unnecessary contrast loads and additional tests, such as MRI or angiography, all of which could provoke considerable anxiety *(133)*. For these reasons, we do not advocate routine screening for RCC in asymptomatic patients with ADPKD.

5. SUMMARY

Screening for RCC has traditionally been hindered by a variety of factors including the relatively low incidence of this tumor in the general population and the lack of well-defined target populations. The prevalence of clinically insignificant lesions, such as renal

adenomas and other benign lesions, such as oncocytomas, would also complicate generalized screening efforts and mitigate against a beneficial effect on the population at large.

At present, screening should be reserved for patients suspected of having one of the familial forms of RCC, and for the immediate relatives of patients known to have one of these syndromes. Intelligent use of molecular analysis and clinical and radiographic monitoring can often provide a definite diagnosis, as well as identify both renal and nonrenal manifestations at a presymptomatic phase, allowing maximal benefit to be achieved from interventive efforts. Radiographic screening should also be considered in patients with ARCD and TS, because these patients also appear to be at an increased risk of developing RCC. In all cases, screening should be pursued on an individualized basis, taking into account patient age and presence of significant comorbidities, and incorporating the preferences of the well-informed patient.

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7 Renal Cell Carcinoma

Diagnosis and Staging

Joel W. Slaton and David A. Swanson

CONTENTS

1. INTRODUCTION

Renal cell carcinoma is the common name applied to adenocarcinoma of the kidney, a tumor arising in the renal cortex and accounting for approximately 85% of malignancies of the kidney *(1)*. Kidney cancer is the third most common malignancy of the urinary tract, after prostate cancer and bladder cancer. The American Cancer Society estimates that there will be 30,000 new cases and 11,900 deaths from kidney cancer in the United States in 1999 *(2)*. The incidence of renal cell carcinoma was previously reported to have increased about 35% over the past two decades, with slightly decreased mortality rates *(1)*. A more recent analysis, however, confirmed the increased incidence rates between 1975 and 1995, but also reported an increase in mortality rates for all race and sex groups *(3)*. Both reports postulated that the increased incidence rates reflect earlier diagnosis at a earlier (and more treatable) stage, largely caused by more frequent and liberal use of modern radiological imaging techniques. Nonetheless, this tumor is still commonly diagnosed late because it is characterized by few symptoms and signs directly related to the renal primary site. It has been called the "internist's tumor" because of its associated systemic effects. In the past, up to 40% of patients already had metastases when the initial diagnosis was made, but this figure does not reflect the contemporary increase in tumors diagnosed incidentally. The median age at presentation is 65 yr, and the ratio of men to women with kidney cancer is approximately three to two (17,800 to 12,200 in 1999, as estimated by the American Cancer Society) *(1,2)*.

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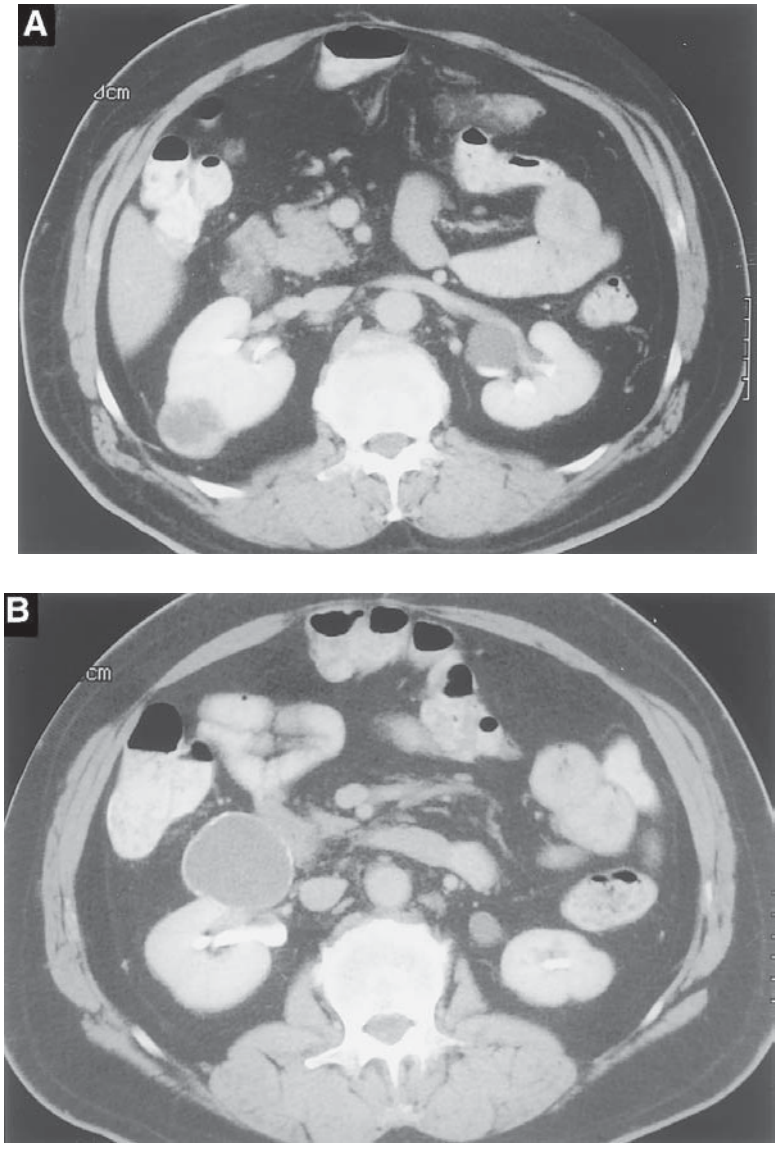

Fig. 1. CT scan of a kidney with two cystic lesions (**A** and **B**) at opposite poles. Although aspiration biopsy of each mass was negative for tumor, nephron-sparing surgery was performed, and renal cell carcinoma was found in each mass.

2. DIAGNOSIS

Renal cell carcinoma is often not diagnosed until after the tumor has become large and/or advanced. However, the tendency in relatively recent years to perform ultrasonography, computed tomography (CT), or even magnetic resonance imaging (MRI) for other indications has led to increased detection of small, localized, asymptomatic tumors *(4,5)*. It was estimated in the mid-1980s that 25–40% of kidney tumors were diagnosed as an incidental finding, compared with less than 10% before to such routine use of radiological imaging. The availability of these newer-imaging modalities also permits more accurate diagnosis of renal tumors, particularly small ones under 3 cm, and the relative merits

of these modalities are discussed by Amendola et al. *(6)*. The use of radiologic imaging modalities for both diagnosis and staging will be discussed later in this chapter.

Hematuria, the most common presenting symptom, occurs in 50–60% of patients *(7)*. Abdominal pain, frequently localized to the flank and caused by bleeding within the tumor or invasion of adjacent organs, is present in 40–50% of patients; about 30% have a palpable mass. This "classic triad" is present in only 10–15% of patients and is a late sign *(8)*. If all three symptoms are present, almost one-half of these patients are likely to have metastatic disease already. Other symptoms and signs are nonspecific and include the paraneoplastic syndromes that are common to this disease and discussed in Chapter 8. Typically, a patient may report fatigue, malaise, anorexia, nausea, fever (often with night sweats), and weight loss. Weight loss, weakness, and anemia were the earliest manifestations in one-third of patients in years past *(9)*. Signs of tumor may include anemia or polycythemia, hypertension, hypercalcemia, hepatic dysfunction (Stauffer's syndrome), and amyloidosis *(8)*. Varicocele, which usually is on the left because the testicular vein drains into the left renal vein and is at increased risk for obstruction, occurred in 0.6% of a large series of 2314 men with renal tumors *(10)*. Manifestations of vena cava obstruction such as varicoceles, recurrent pulmonary emboli, proteinuria, lower extremity thrombosis, or edema are present in only 36 to 50% of patients with vena cava thrombus because of collateral venous drainage through the lumbar and azygos systems *(11,12)*.

2.1. Biopsy

It is not necessary or advisable to routinely perform percutaneous needle aspiration or core biopsy of a solid renal mass before surgery. The reason is not because of significant risk of seeding of the biopsy tract, because that has occurred extremely rarely, but because of the risk of a false-negative biopsy (Fig. 1). Furthermore, such a biopsy is not usually helpful for treatment planning. Because most solid renal masses are malignant, there are only four reasons to establish the diagnosis by biopsy. One reason would be to evaluate a patient for whom there is clinical suspicion of an abscess or infected cyst. The second would be to establish that the tumor is benign or of such low-grade malignancy that nephrectomy is not necessary (e.g., in the case of oncocytoma). Unfortunately, the risk of sampling error does not permit us to *conclude* from a biopsy core that a solid tumor is an oncocytoma, only that a portion of the tumor, at least, has oncocytic features. Perhaps cytogenetic typing someday will be so conclusive that it will allow us to sample such a tumor and plan therapy on that basis, but that time has not come yet *(13)*. The third reason to obtain a diagnosis by biopsy would be to confirm the presence of a malignancy that might be treated successfully by chemotherapy instead of radical nephrectomy. This includes patients with suspected metastases to the kidney from another known malignancy. Also, patients with lesions that appear to *infiltrate* the kidney and adjacent structures, as typically seen with lymphoma (Fig. 2) and transitional cell carcinoma (TCC) of the kidney (Fig. 3), for example, should undergo biopsy before planned surgical removal of the kidney. Although many patients with TCC of the kidney ultimately require nephrectomy, initial chemotherapy might be better for the patient with advanced disease because it is tolerated better by the patient with two functioning kidneys. Finally, biopsy might be warranted in the patient with extensive metastatic disease for whom the surgeon is contemplating nephrectomy. If the biopsy were to reveal high-grade tumor, particularly a sarcomatoid tumor, the poor prognosis associated with such a finding might be sufficient reason to cancel the planned nephrectomy.2

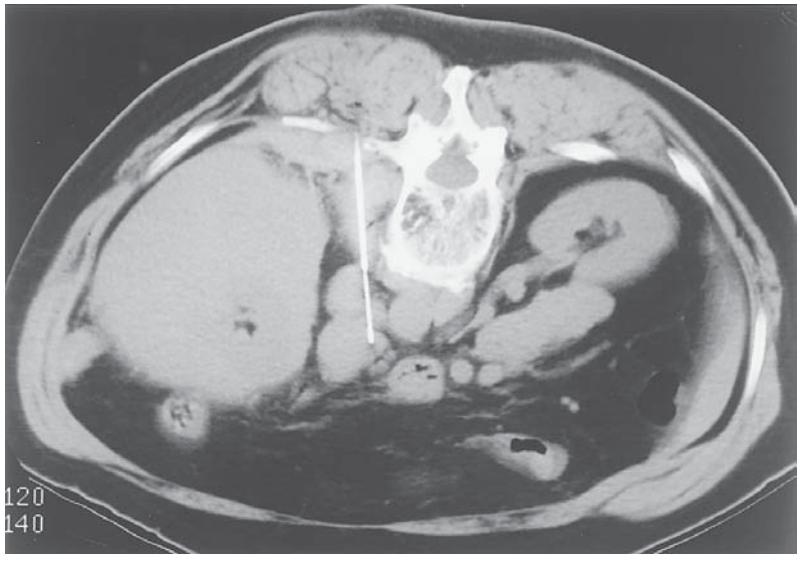

Fig. 2. CT scan of an infiltrative process involving the left kidney in a patient with enlarged paraaortic lymph nodes. Biopsy of a lymph node (shown) and renal mass both revealed malignant lymphoma.

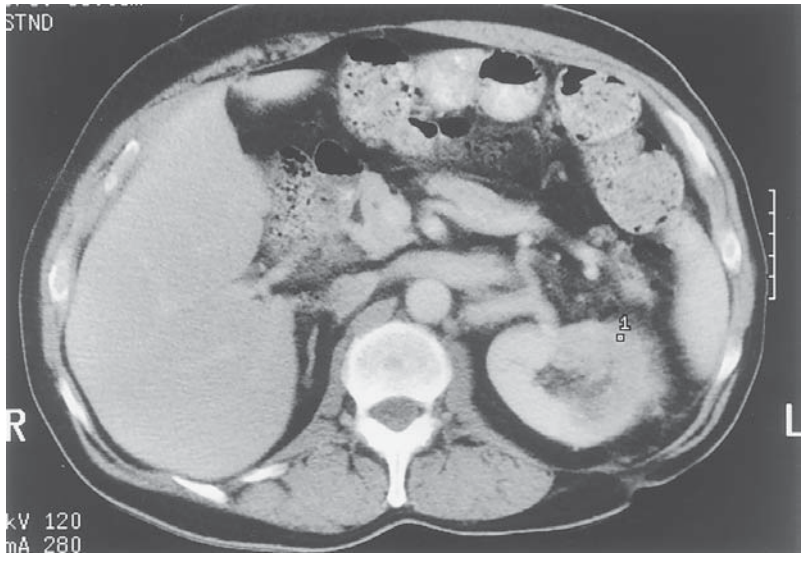

Fig. 3. CT scan of an infiltrating lesion in the left kidney. Biopsy confirmed the presence of high-grade transitional cell carcinoma invading the parenchyma.

2.2. Differential Diagnosis

Attempts to distinguish different cellular subtypes of renal cell carcinoma by radiographic imaging have generally been unsuccessful. This has been particularly true for renal oncocytoma, whose "spokewheel" appearance on arteriogram and stellate central scar were formerly thought to be specific. However, it is now believed that all of the findings that were formerly believed to distinguish these subtypes can be found in all subtypes of renal cell carcinoma and that CT scans and MR imaging cannot reliably distinguish these subtypes of tumors *(14,15)*.

The presence of fat seen on CT or ultrasonography is pathognomonic of angiogmyolipoma, a benign lesion made up of vasculature, smooth muscle, and fat *(16)*. Occasionally, the fat content is low and may be revealed only with thin sections and measurement of the density in Hounsfield units. The diagnosis is more reliable in patients with other stigmata of tuberous sclerosis, because rare renal adenocarcinomas have been shown on CT scan to contain fat *(17,18)*. Unfortunately, cases of coincidental existence of angiomyolipoma with renal adenocarcinoma and renal oncocytoma have both been reported *(19,20)*.

Renal lymphoma has a broad spectrum of manifestations upon imaging. Typical findings include solitary or multiple nodules (unilateral or bilateral), diffuse infiltration, and diffusely enlarged retroperitoneal lymph nodes *(21)*. Cohan and associates reported that 59% of patients with lymphoma and renal involvement had bilateral renal disease *(22)*. Of these patients with bilateral renal disease, only 40% had associated enlarged retroperitoneal nodes, 10% had infiltration of the perirenal space without any significant renal parenchymal involvement, and only 3% had a solitary renal mass.

TCC has been discussed with regard to possible biopsy. TCC is hypovascular, but so are some renal cell carcinomas. If the tumor infiltrates the renal parenchyma, it may look more like a renal cell carcinoma than a renal pelvic or calyceal tumor. Any patient with a central lesion should be considered, at least, to have TCC, and retrograde pyelography and urinary cytology (with or without biopsy) may help establish the correct diagnosis.

Metastatic lesions in the kidney are also generally hypovascular and frequently multiple, although a solitary lesion may be a metastasis (Fig. 4). The primary tumors most likely to metastasize to the kidney are melanoma and tumors of the breast, lung, intestine, stomach, ovary, cervix, pancreas, uterus, and prostate *(23,24)*.

Nonmalignant conditions may appear as a renal mass. Clinical evaluation and optimal radiologic evaluation may help distinguish renal cell carcinoma from a renal infarct, a pseudotumor caused by a hypertrophied column of Bertin, an abscess, or a vascular lesion such as an arteriovenous fistula (Fig. 5).

3. STAGING

The staging evaluation has two goals. The first goal is to determine whether there is metastatic disease, which might have direct bearing on whether to proceed with management of the primary tumor immediately or on a delayed basis. The second goal is to determine the local extent of the tumor in order to plan the best surgical procedure and approach for management of the primary tumor, as well as to plan any associated procedures that might be required.

The staging work-up seeks to evaluate the sites at highest risk for metastases. As determined by autopsy, the most common sites of metastases in patients with renal cell carcinoma are the lungs (with such metastases present in at least 50% of the patients with metastases), lymph nodes (in about 35%), liver (in about 30%), bone (in about 30%), brain (in about 5%), adrenal (in up to 5%), and contralateral kidney (in 1–2%) *(7,23)*. However, it is important to remember that this tumor also spreads to such very unusual sites as heart and pericardium, spleen, intestine and mesentery, skin, diaphragm, pancreas, thyroid, ureter, epididymis, skeletal muscle, gallbladder, penis, urinary bladder, and ovary.

This diverse presentation of metastases should guide the staging evaluation, which should begin with a complete medical history and careful physical examination. Specific complaints of pain or organ dysfunction might point the alert clinician toward a specific metastatic site, and a history of significant weight loss is often an ominous clue that

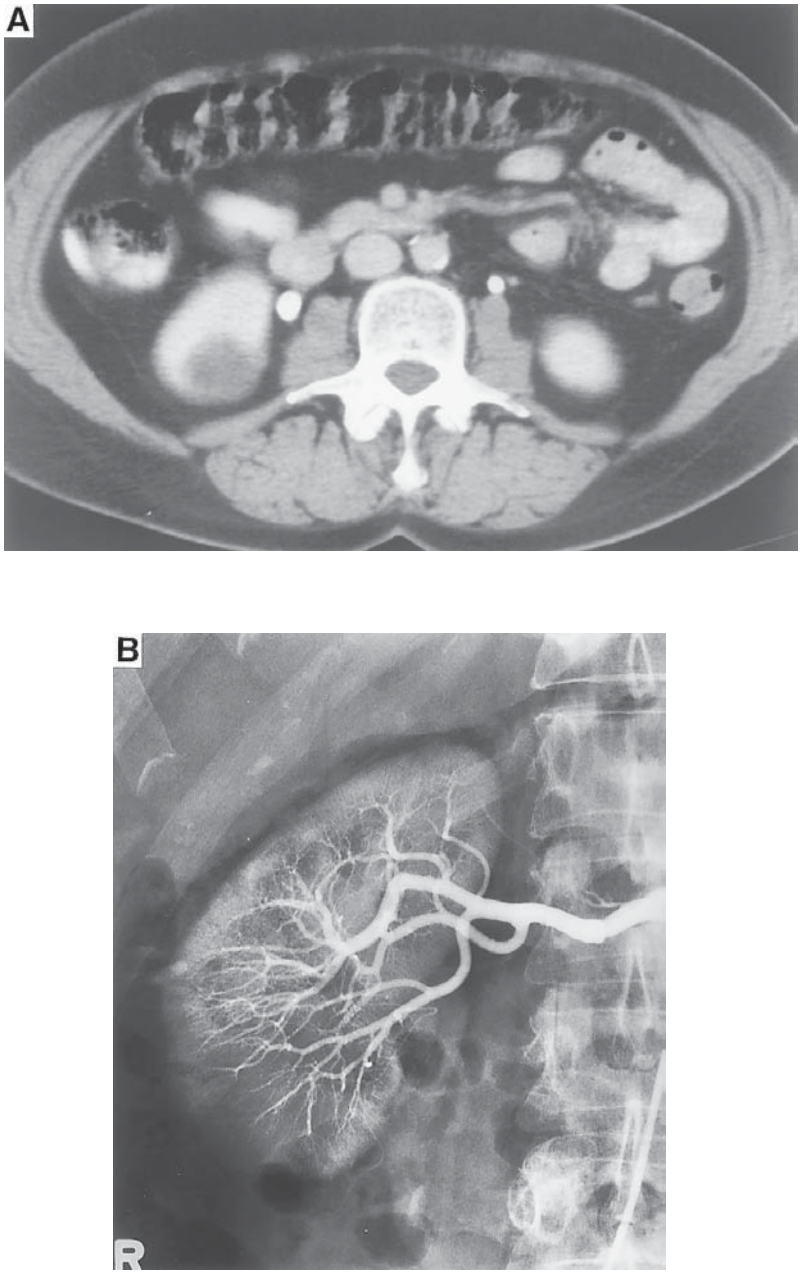

Fig. 4. CT scan (**A**) and arteriogram (**B**) of a solid but hypovascular mass.

should prompt the physician to be especially vigilant in looking for occult metastases. For the patient with a history of gross hematuria, cystoscopy is recommended to ensure there is no metastasis in the bladder.

The physical examination might disclose frank tumor, such as a metastatic lesion in the scalp or skin. Palpation may reveal a mass or a focus of tenderness that suggests the presence of an underlying metastasis. It is important to palpate the supraclavicular area

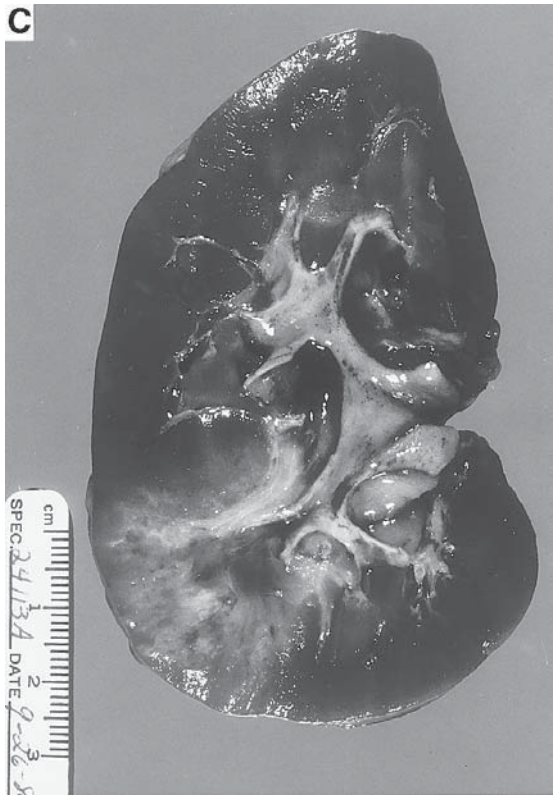

Fig. 4. (Continued). (**C**) This mass was proved by nephrectomy to be metastatic breast cancer.

very carefully for enlarged lymph nodes, because this is a common site of nodal metastases. This evaluation should be performed both while the patient is supine and while the patient is sitting up, because sometimes it is possible to palpate subtly enlarged nodes in one position and not the other. A neurological examination may disclose signs that lateralize or a cranial nerve palsy that points to a central lesion.

The radiographic evaluation must also take into consideration the most common sites of metastases, and the unusual sites should not be forgotten during the interpretation of the X-rays and scans. First and foremost of these tests is the chest X-ray. Posterior-anterior and lateral views are usually sufficient. It is most cost-effective to reserve CT of the chest for evaluation of indeterminate findings or sometimes for the patient determined to be at particularly high risk for occult metastases or who is facing a particularly complex surgical procedure that is justified only in the absence of metastases. The abdominal viscera and lymph nodes should be assessed with a CT scan or MRI of the abdomen and pelvis, but these modalities should be used to evaluate the brain only in the patient with clinical findings that suggest the possibility of metastases or in whom the presence of metastases at other sites increases the risk of brain metastases as well. Routine use of bone scans is no longer recommended for the asymptomatic patient whose serum alkaline phosphatase and calcium concentrations are both normal *(25,26)*. However, in the patient who has clinical findings that strongly suggest the possibility of osseous metastasis and in whom the bone scan was performed but was normal, the evaluation

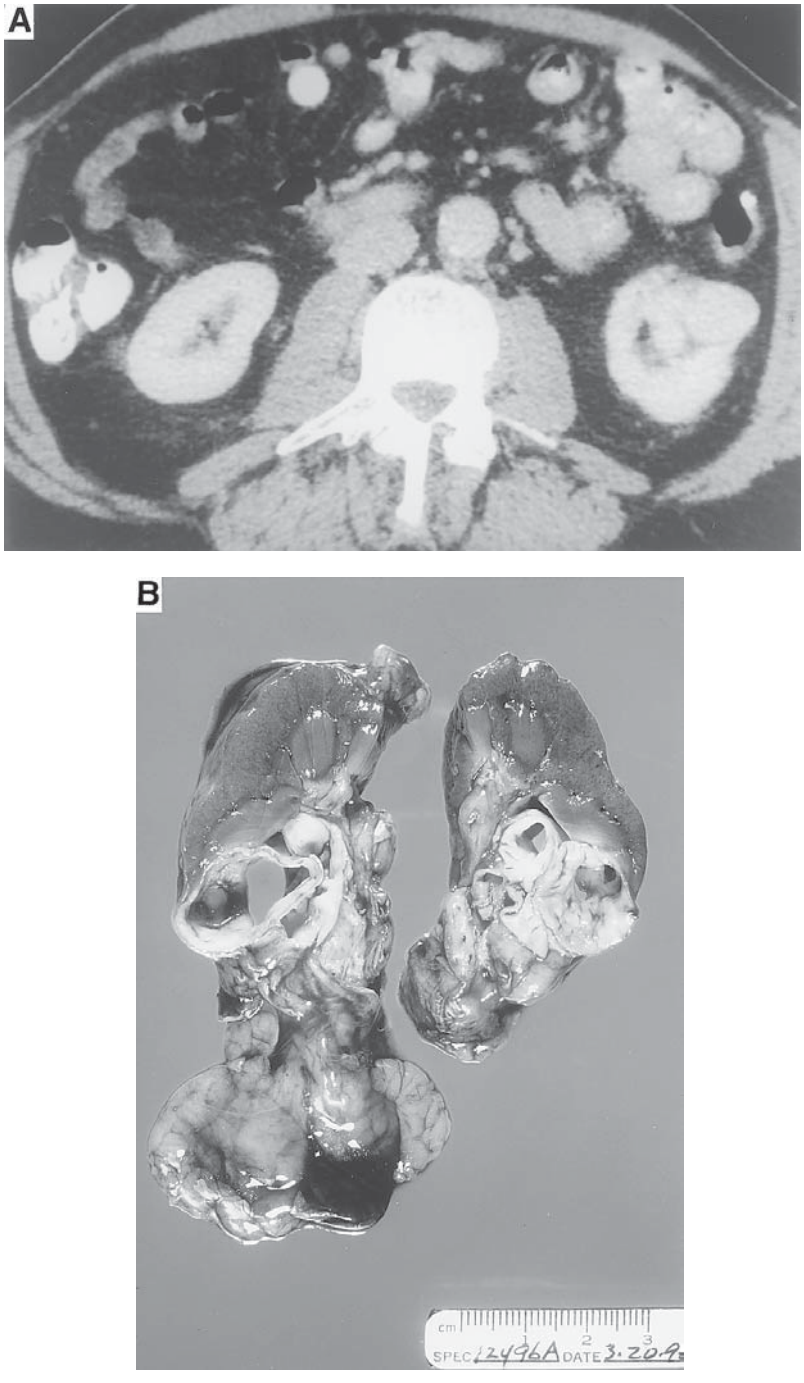

Fig. 5. CT scan (**A**) of an apparently solid parenchymal mass in the left kidney in a patient who had previously undergone percutaneous nephrolithotomy for stones. Nephrectomy (**B**) revealed this to be an arteriovenous fistula.

should continue. The bone scan usually shows absence of uptake for lytic metastases, which is typical of renal cell carcinoma and not particularly sensitive. Plain X-rays, too, may fail to reveal even large lytic lesions, particularly in the pelvis. For the patient with

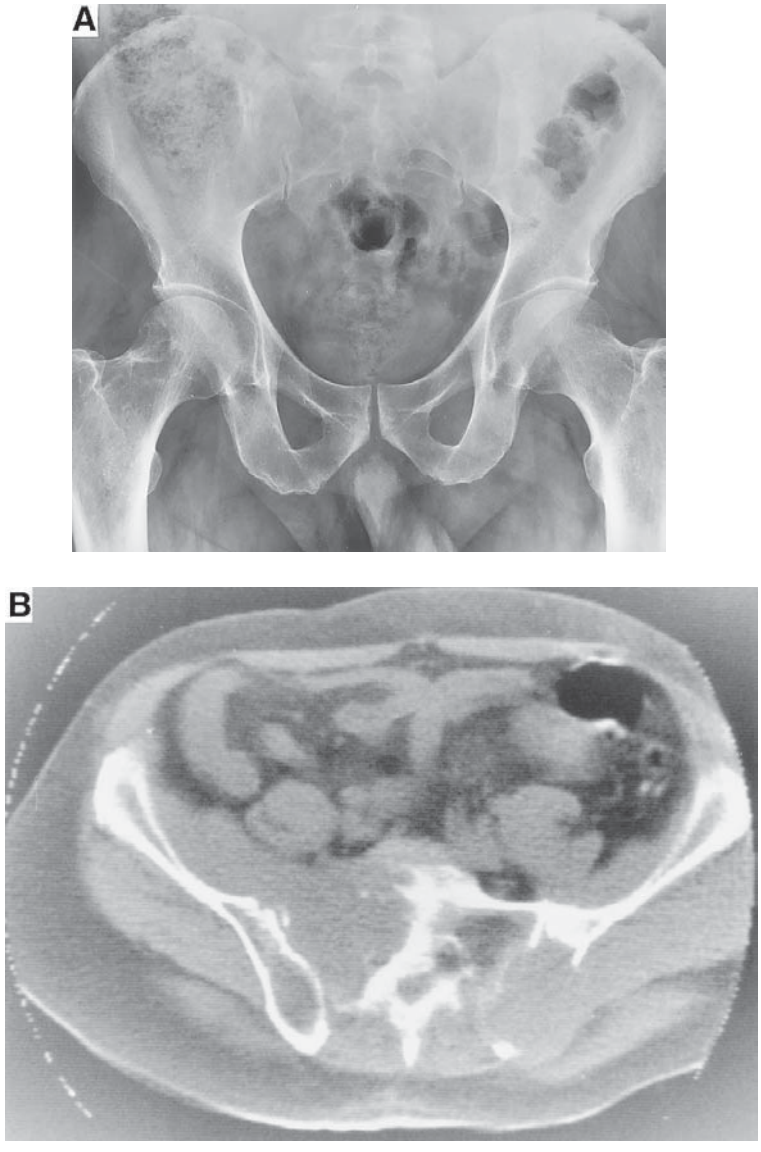

Fig. 6. Plain X-ray (**A**), interpreted as normal, of a patient complaining of pain in his pelvis. A CT scan (**B**) showed a lytic metastasis with an associated soft tissue mass.

likely, but still undiagnosed, osseous metastasis, a CT scan or MRI may reveal both the lytic lesion and possibly an associated soft-tissue mass (Fig. 6) *(28)*.

Another purpose of the staging evaluation is to look for possible tumor markers. Although abnormal results for blood tests may lead to a more vigorous search for metastases, such tests may also be potentially helpful as nonspecific markers after therapy. For instance, even serum hemoglobin might serve as a marker if the patient presents with anemia or polycythemia. Following surgical extirpation of all disease, the hemoglobin level may become normal, but if it reverts to the preoperative level later, this may herald recurrence. In addition to the hematologic survey, other serum tests that should be performed include creatinine, alkaline phosphatase, liver function tests, and calcium. Serum

calcium concentration is a more common tumor marker than hemoglobin, and after treatment calcium should fall to normal levels and remain normal.

4. RADIOGRAPHIC IMAGING

Staging evaluation of the primary tumor is primarily radiographic. There are many different imaging modalities that provide important information about the nature and extent of the primary tumor, some of it complementary, much of it redundant if multiple studies are performed. Which studies are necessary and how many need to be performed depend to some extent on which study is performed first and on the size and characteristics of the tumor. For example, if a CT scan or MRI is the initial study performed, it may not be necessary to perform any additional studies to evaluate the primary tumor. However, if a renal mass lesion is identified on a plain X-ray of the abdomen or on intravenous urography, additional radiographs will be required to fully evaluate the tumor and to complete the staging evaluation. We will discuss each imaging modality individually. Bechtold and Zagoria have published an excellent and detailed discussion of the advantages and disadvantages of ultrasound, CT, and MRI for evaluation of each clinical stage of renal cell carcinoma *(29)*.

4.1. Excretory Urography

Excretory urography, also known as intravenous urography (IVU) or intravenous pyelography (IVP), continues to be a commonly used initial radiographic evaluation for the patient who presents with gross hematuria. Basically, IVU potentially identifies the cause of the hematuria and permits selection of the patient who needs more specialized modalities for better evaluation. Plain films of the abdomen with tomographic cuts of the kidney prior to injection of the contrast agent are an essential part of the correctly performed IVU. These films may reveal a soft tissue mass in the region of the kidney or distortion of the contour of the kidney itself. In a large series from the Mayo Clinic, calcifications were present in 10% of renal cell carcinomas, and at least 52% of all renal masses with foci of calcification were renal cell carcinomas *(29)*. Central punctate or dense calcifications were associated with renal cell carcinoma in 87% of cases, while rim-like calcification indicated benign renal cysts in 80%.

For IVU to detect the presence of a renal mass, the mass must appear as an irregularity of the otherwise smooth contour of the kidney or must distort the renal pelvis or calyceal system by extrinsic compression. To do so, however, requires the mass to be large enough or located fortuitously where this happens. Although Warshauer et al. reported that 85% of lesions at least 3 cm in diameter were detected by IVU with linear tomography, the detection rates were only 52% for lesions at least 2 but less than 3 cm, 21% for lesions at least 1 but less than 2 cm, and 10% for lesions less than 1 cm *(30)*. Ultrasonography, CT, or MRI will determine whether the lesion seen is cystic or solid. The disadvantage of performing ultrasonography next is that if it is a solid lesion, either a CT scan or MRI will still be required to complete the staging evaluation before surgery.

4.2. Ultrasonography

Transabdominal ultrasonography may lead to the incidental discovery of a renal mass in the patient being evaluated for possible cholelithiasis, in the patient with a pelvic abnormality in whom the technician also evaluates the abdomen as a matter of routine,

or in the patient with microscopic hematuria and negative cystoscopy. The advantages of ultrasonography are its ready accessibility, low cost, non-invasive nature, and the fact that it requires no contrast *(26,28)*. The disadvantages are that its sensitivity is very operator dependent and that the results are influenced by a number of factors such as the presence of intestinal gas and the patient's body habitus. The sensitivity of ultrasonography is similar to that of IVU with linear tomography as described above *(30)*. Ultrasonography is particularly good for determining if a renal mass lesion is solid, and therefore presumed to be tumor, or a simple or complex cyst. The ultrasound criteria required for the diagnosis of a simple cyst are (1) thin, sharply defined smooth walls, (2) good though-transmission with acoustic enhancement of the posterior wall, and (3) the absence of internal echoes. Such simple cysts are Bosniak I lesions and are defined by either ultrasonography or CT (the Bosniak classification will be discussed in detail in the section on CT) *(31)*.

Doppler-enhanced ultrasonography is a potential improvement because it can identify tumor neovascularity, but 20% of renal cell carcinomas are not vascular. Doppler ultrasonography facilitates the assessment of tumor thrombus extension into the renal veins, inferior vena cava, and the right side of the heart *(32)*. The sensitivity has been reported to range from 70 to 81% and the specificity from 94 to 98%.

Intraoperative ultrasonography has become popular recently as an adjunct to nephron-sparing surgery *(33,34)*. It helps to define the precise location and depth of the lesion and to survey the rest of the kidney for previously unrecognized solid lesions. When color Doppler ultrasonography is also used, it permits identification of arteries, veins, and the collecting system near the potential resection site and helps to define the plane of dissection between tumor and vital vascular structures *(35)*. Polascik and associates recently reported on 100 consecutive patients who underwent intraoperative ultrasound *(36)*. Eight patients who were considered candidates for partial nephrectomy underwent radical nephrectomy because intraoperative ultrasonography revealed more extensive tumor, and three patients with suspected malignancy were spared nephrectomy after ultrasonography and frozen section analysis revealed benign cysts.

Intraoperative ultrasonography may also help assess tumor thrombus in the inferior vena cava in patients with inconclusive preoperative studies or unexpected intraoperative findings *(37)*. It can establish the presence or absence of thrombus and its extent, especially when these factors are not definitively established preoperatively. Transesophageal echocardiography, (TEE) is an excellent way to intraoperatively assess the superior extent of the tumor thrombus, particularly when atrial extension is present, and potential sites of wall invasion and/or residual disease after thrombectomy *(38)*. Both transabdominal ultrasonography and TEE can be used, of course, for the preoperative assessment of vena cava thrombus.

4.3. Angiography

Although about 80% of renal cell carcinomas are hypervascular and have a typical tumor blush after arterial injection of a contrast agent, the introduction of new imaging modalities has substantially reduced the value of angiography in the management of renal cell carcinoma *(39)*. Blood vessels to and within the kidney can now be seen well with either modified CT or MRI techniques. Also, a negative angiogram does not exclude the presence of renal cell carcinoma, and an apparent "avascular mass" on angiography may be enhanced on CT *(31)*. Some surgeons still prefer to obtain a diagnostic arteriogram

before nephron-sparing surgery, but it is rarely necessary for other than a large, central lesion, and newer three-dimensional reconstructions of CT scans may render even this use obsolete when the newer technique becomes widely available *(40,41)*.

The one remaining use for arteriography is renal artery occlusion (embolization or angioinfarction). Although this procedure is not used often, some surgeons do employ it in patients with tumor thrombus in the inferior vena cava in an attempt to shrink the thrombus preoperatively *(42,43)*. Because these thrombi are frequently arterialized, angioinfarction can make them retract. We have seen atrial thrombi retract into the suprahepatic vena cava below the diaphragm, and suprahepatic thrombi retract well below the entrance of the hepatic veins, greatly facilitating thrombectomy.

4.4. Venacavography

For the most part, more modern imaging modalities have supplanted the time-honored use of venacavography to evaluate the inferior vena cava for tumor thrombus. However, Horan et al. reported in 1989 that the combination of venography and MRI resulted in higher diagnostic yield than either test alone, even though the diagnostic accuracies of each used alone were essentially equal *(44)*. In general, CT and MRI have replaced cavography for the identification of tumor in the renal vein and inferior vena cava *(28,45,46)*.

4.5. Computerized Tomography

Computerized tomography is still the next imaging modality likely to be used after the diagnosis of an apparent solid renal mass by IVU or ultrasonography because of its ready availability, low cost, and high accuracy for the diagnosis and staging of renal cell carcinoma *(28,30)*. Also, regardless of what imaging test first indicated a solid renal mass, all patients should undergo either a CT scan or an MRI as the best way both to evaluate the primary lesion and to exclude metastatic disease. As early as 1980, CT was reported to have a diagnostic accuracy of better than 95% for renal cell carcinoma *(39)*. Current technology permits helical scanning, which completes the entire scan during a single breathhold and minimizes motion artifact *(28)*. Scans through the kidney are typically taken at 5-mm intervals, although thinner sections may be obtained to assess very small lesions (Fig. 7). Helical, or spiral, CT is a major advance that allows true volumetric rather than sequential single-slice data acquisition *(47,48)*. Axial images from any level can be reformatted in other planes or even in three dimensions (Fig. 8). Scans are taken before and after injection of the contrast agent with a power injector, enabling assessment of the kidney during the arterial phase and the later venous phase as the renal vessels are opacified. This ability to perform CT angiography has led to new applications, such as that reported recently from the Cleveland Clinic in which patients who were surgical candidates for nephron-sparing surgery underwent preoperative triphasic spiral CT with subsequent three-dimensional reconstruction *(41)*. The three-dimensional volume-rendering CT accurately depicted renal parenchymal and vascular anatomy in a format familiar to surgeons and integrated essential information traditionally obtained from angiography, venography, excretory urography, and conventional two-dimensional CT into a single imaging modality.

If helical CT scanning is not available for staging, conventional CT should be performed in a dynamic fashion, which has been shown to be superior to nondynamic CT scans, ultrasonography, and arteriography in assessing extracapsular spread, or renal vein or inferior vena cava invasion *(49)*.

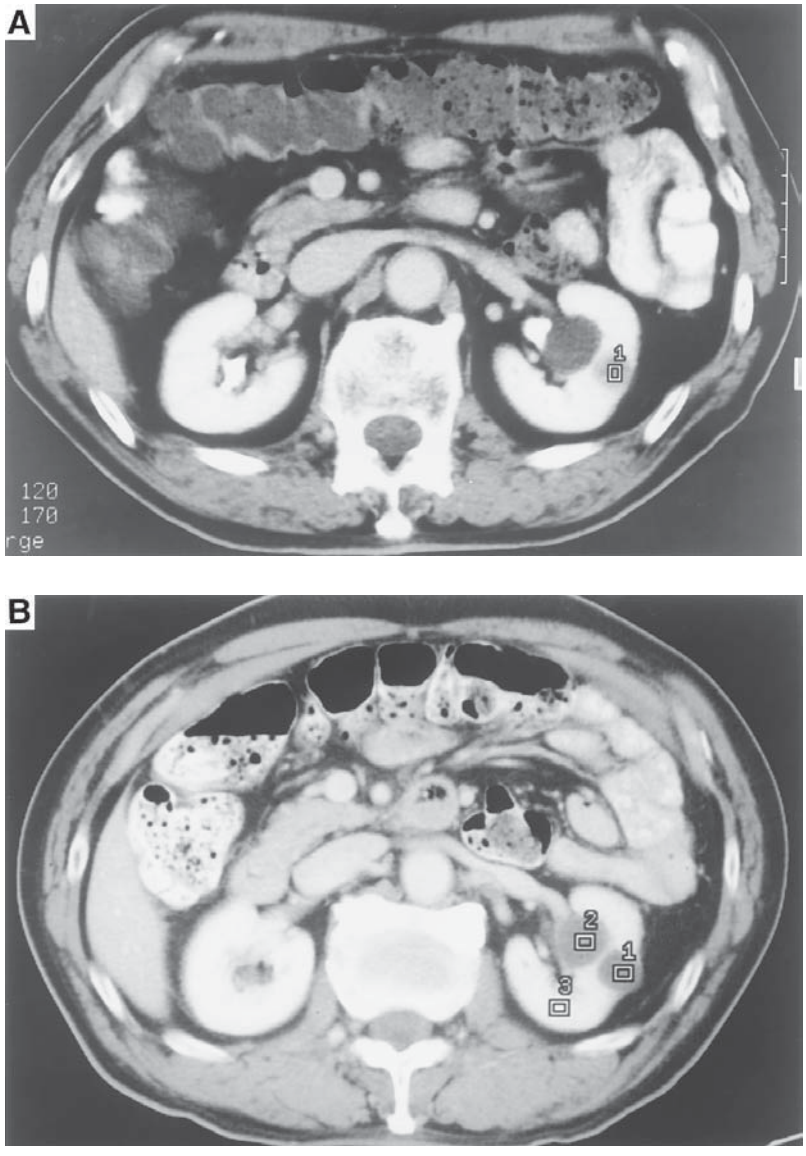

Fig. 7. Conventional CT scan (**A**), interpreted as showing a small, enhancing, parenchymal mass with a density of 54.9 Houndsfield units (HU). When the procedure was performed as a helical CT (**B**), only the normal parenchyma was enhanced after contrast (208.3 HU); the larger parapelvic cyst had a density of 1.0 HU, and the small parenchymal cyst originally thought to be enhanced had a density of only 5.3 HU.

Computed tomography need not be used to confirm the diagnosis of a classic renal cyst diagnosed by ultrasonography. However, it remains the gold standard for the characterization of an indeterminate renal mass. Bosniak has proposed a classification of cystic renal masses based on ultrasonographic and/or CT evaluation *(31)*. Bosniak category I cysts are classic, uncomplicated, simple benign cysts that are well marginated and the same density as water; they show a thin wall and have no contrast enhancement. Although there is at least one report of a Bosniak I cyst that contained tumor (although the classification

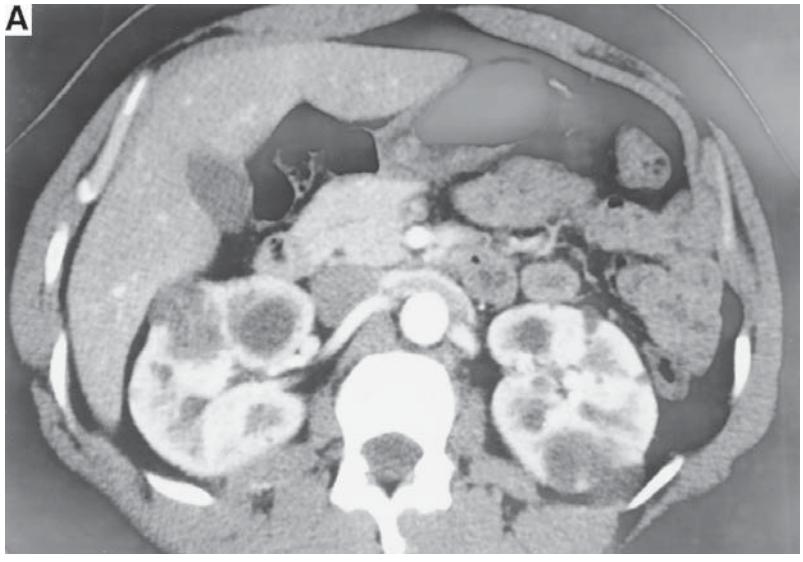

Fig. 8. Helical CT scan of a patient with adult polycystic kidney disease, showing various images of an enhancing cystic lesion. (**A**) Standard image (axial projection), showing multiple cysts and the renal cell carcinoma.

by ultrasonography was a Bosniak IV) *(50)*, for all practical purposes a Bosniak I cyst should be considered benign when defined properly, and such a cyst does not require surgical removal (or exploration) *(51,52)*.

Bosniak category II cysts are minimally complicated and for the most part benign, but they have some radiologic findings that cause concern. These lesions may show a few thin septations or calcifications, or they may be "hyperdense cysts." Strictly defined, a hyperdense cyst must have a homogeneous pre-contrast Hounsfield density greater than 20 and *no enhancement after contrast*; it must be round and smaller than 3 cm in diameter and have one-quarter of its circumference outside the renal outline *(53)*. Malignant lesions have also been reported in Bosniak II lesions, but most of those diagnoses have not been made by a strict renal CT protocol *(54)*. Nonetheless, it is clear that some category II lesions will contain cancer, even if it is only in 10–15% of such cysts, and the urologist and radiologist must exercise careful clinical judgement regarding surgical removal. For the most part, however, Bosniak II cysts are considered not to require surgery when strictly defined by an appropriately performed CT scan, but patients should have follow-up imaging performed every 6 to 12 mo.

Bosniak category III cysts are more complicated cystic lesions. They may show thick, irregular, or nodular walls, thick or irregular calcifications or septations, or multiple septations; above all, they do not meet strict criteria for a Bosniak II cyst. At least 40% of Bosniak III lesions will contain cancer *(51,52,55)*. Bosniak category IV lesions are malignant appearing with large cystic components. They have an irregular, thickened wall or nodularity in the wall or a solid component. Enhancement of the cyst, cyst wall, or any septum or nodule may be present. These category IV lesions should be considered malignant until proved otherwise, because they will be so in at least 90% of cases *(51,52,55)*. It should be clear from the above that all Bosniak III and IV lesions and some Bosniak II cystic lesions should be surgically removed. The decision for nephrectomy

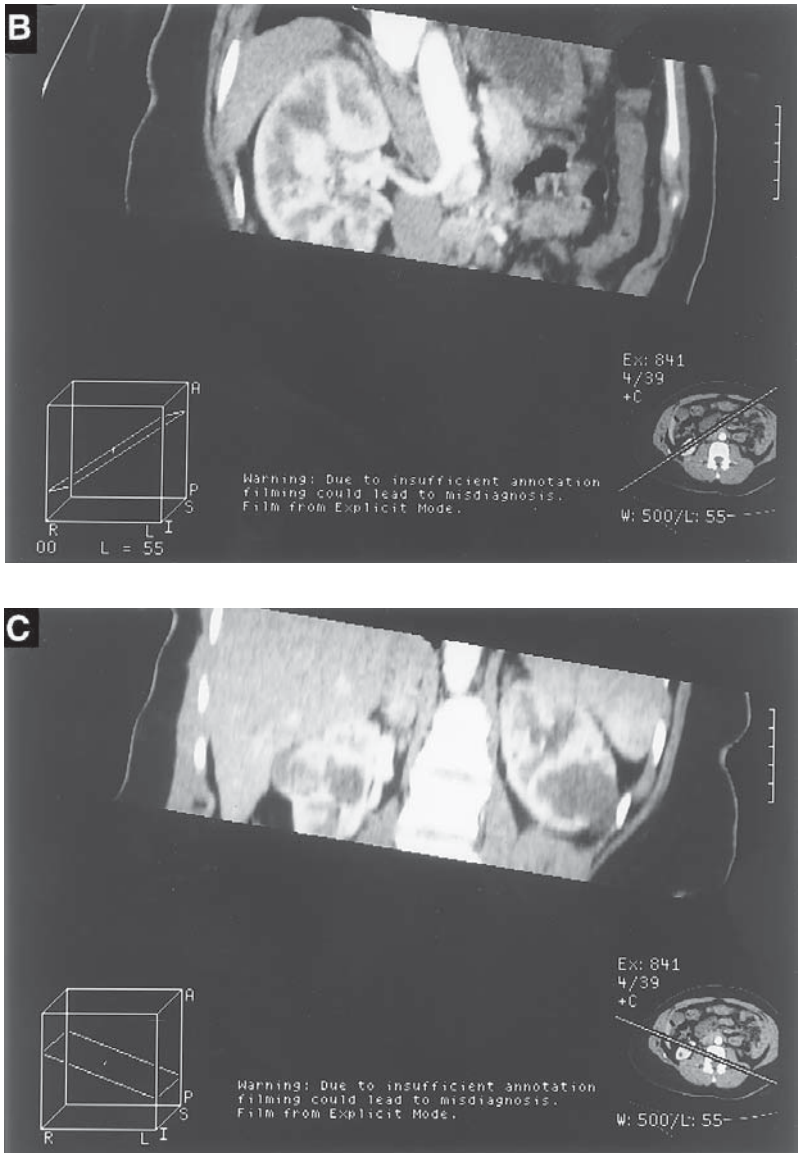

Fig. 8. (Continued). (**B**) and (**C**) angled, reformatted images.

(or, in selected cases, nephron-sparing surgery) should be made before surgery starts. We believe that "exploration" of the cyst, or unroofing and close inspection—with or without biopsies—should *not* be performed because of the potential for false–negative inspection or biopsy and tumor spillage. Observation should be safe for well-defined Bosniak I lesions and is recommended.

Computed tomography may also show enlarged lymph nodes. While it is well known that normal-size lymph nodes may contain microscopic tumor, it may not be fully appreciated that moderately enlarged lymph nodes may or may not contain cancer (Fig. 9) *(56)*. In fact, if enlarged lymph nodes are the *only* reason for not considering surgery because metastatic disease is suspected, needle aspiration biopsy should be performed. Sometimes,

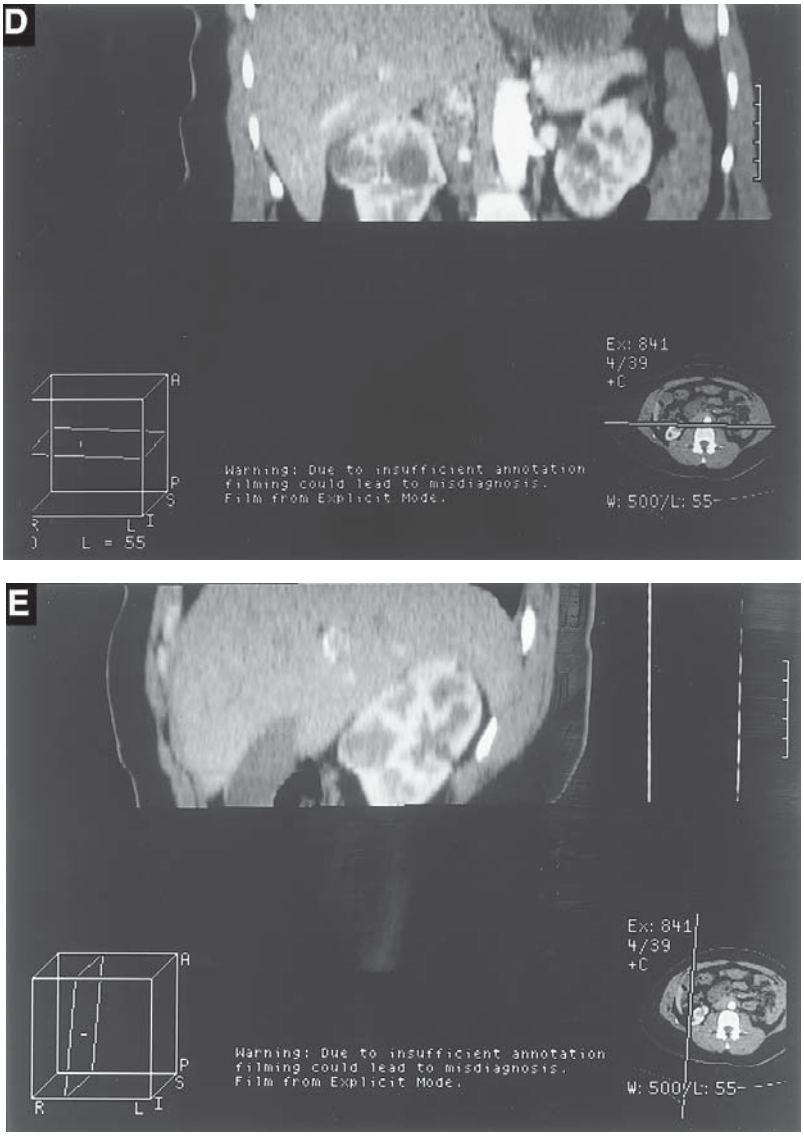

Fig. 8. (Continued). (**D**) horizontal image (coronal projection); (**E**) vertical image (sagittal projection).

enlarged lymph nodes cannot be distinguished from adjacent vascular structures. Magnetic resonance imaging is an excellent modality for making this differentiation *(28)*.

4.6. Magnetic Resonance Imaging

Magnetic resonance imaging is commonly used following CT if there are still questions related to diagnosis or stage of the tumor, particularly whether there is involvement with associated organs *(28,57,58)*. Despite the introduction of the paramagnetic contrast agent gadolinium–DTPA (diethylenetriaminepentacetic acid), which permits tumor enhancement similar to that of contrast-enhanced CT, the superior resolving power of CT and its lower cost mean that CT remains the primary imaging modality for renal mass characterizations *(26,59,60)*. The most common use for MRI of the kidney is staging,

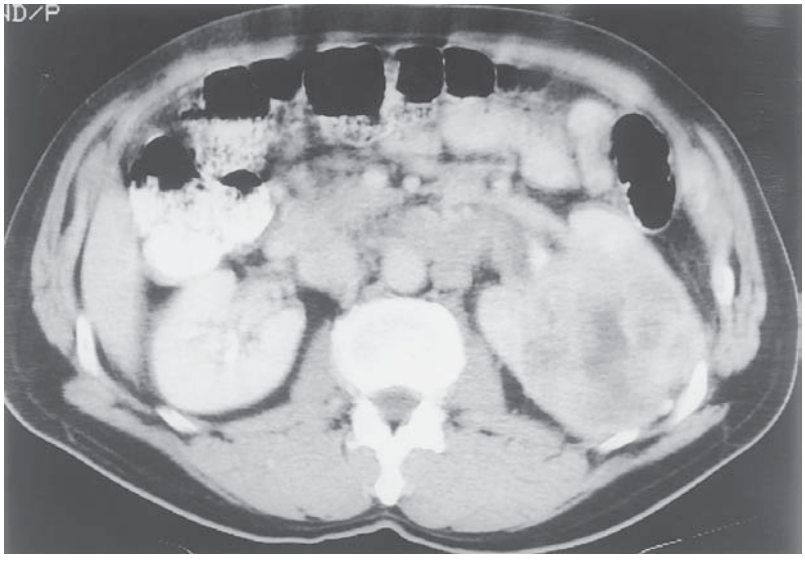

Fig. 9. CT scan of enlarged paraaortic lymph nodes proved by lymphadenectomy to be free of tumor.

particularly the detection of tumor extension into the inferior vena (Fig. 10) *(45,46)*. Horan et al. prospectively compared MRI with vena cavography in 44 patients, and the two modalities had comparable sensitivities and specificities *(44)*. Hricak et al. showed that 12 of 15 patients with caval involvement were accurately staged, with only one patient being understaged *(61)*. In another study, 13 of 13 patients with inferior vena cava thrombus and 23 of 26 patients with renal vein involvement were correctly identified using gradient recalled echo MRI *(45)*.

The advantages of MRI include the fact that it does not require an iodinated contrast agent, making it the imaging modality of choice for the patient who has a contrast agent allergy or renal insufficiency. It also provides images in sagittal, coronal, and oblique projections, which are not available with conventional CT scanning (although helical scans can do so) *(28)*. The ability to distinguish nodal or other soft tissue masses from vascular structures without intravascular contrast is also a benefit. The disadvantages, however, are numerous. Time and expense may limit accessibility of MRI, and some patients may not fit, or be too claustrophobic to lie within, the gantry. Also, patients with pacemakers, intracranial aneurysm clips, or loose metal fragments are not candidates for MRI.

4.7. Positron Emission Tomography

Positron emission tomography (PET) is a new form of imaging that allows imaging of neoplasms through the noninvasive measurement of in vivo biochemical and physiological reactions *(62,63)*. The glucose analogue 18-fluoro-2-deoxyglucose (FDG) is the most common radiotracer used. High uptakes of FDG are normally seen in the kidney because of the inability of the nephron to reabsorb filtered FDG in the convoluted tubules.

Relatively few studies have applied FDG PET to renal cell carcinomas. Bachor et al. studied 29 patients who were scanned before resection of a primary tumor *(64)*. The PET scan identified histologically confirmed renal cell carcinoma in 20 patients but was unhelpful in six cases. In three patients with benign lesions, the PET scan was falsely positive, while in another three patients it was able to detect regional lymph node metastases. Miyauchi

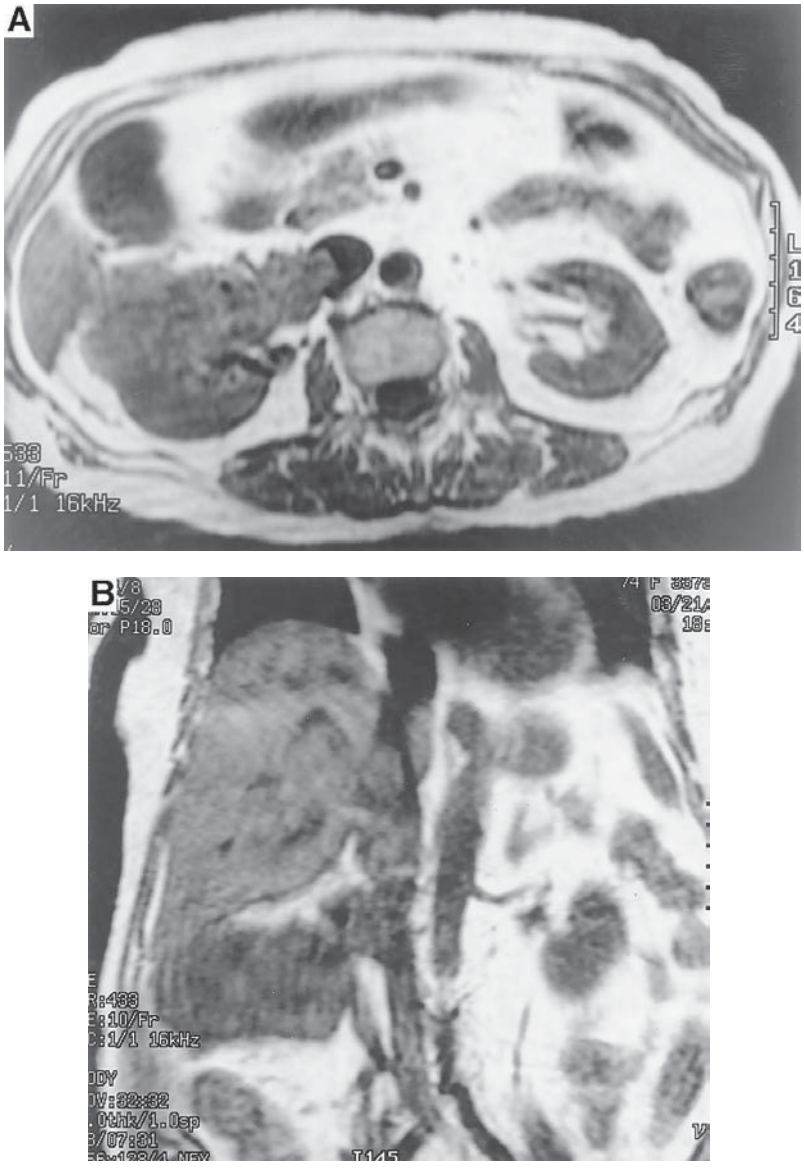

Fig. 10. MRI of a patient with tumor thrombus in the inferior vena cava (IVC). Transverse projection (**A**) showed thrombus projecting into the lumen of the IVC. Coronal projection (**B**) defined the cephalad extent of the thrombus.

et al. demonstrated that patients with a positive PET scan had higher grade tumors than patients with a negative PET scan, although there were no significant differences in intensity of PET based on variables such as tumor size *(65)*.

In 22 patients who had metastatic disease and who were being treated with interleukin-2, PET detected 10 lesions that were actively growing, whereas CT detected only seven growing lesions *(66)*. In five patients with a partial response to treatment, whole-body PET was negative in three cases and mildly positive in two, while conventional imaging showed no changes (three cases) or decreasing tumor size (two cases). Whole-body PET

Table 1
Robson's Classification of Renal Cell Carcinoma

Stage I:	Tumor is confined to the kidney parenchyma with an intact renal capsule.
Stage II:	Tumor has invaded through the renal capsule into perirenal fat, but the tumor is still confined within Gerota's fascia.
Stage III A:	Renal vein or inferior vena cava is grossly involved.
Stage III B:	Lymphatic involvement.
Stage III C:	Venous and lymphatic involvement.
Stage IV A:	Tumor involves adjacent organs other than the adrenal gland.
Stage IV B:	Distant metastasis.

was negative in all five patients with absence of disease or with complete response. Other studies have confirmed these results *(67)*. Thus, PET may be another method of monitoring response to therapy, although further study is needed to determine its ultimate role in staging patients *(68)*.

4.8. Radiolabeled Monoclonal Antibody Scans

Although clinical utility has not yet been proven, it is also possible to image renal cell carcinoma (both primary tumor and metastases) with ^{131}I-labeled monoclonal antibody G250, as reported by Oosterwijk and associates *(69)*. G250 is a cell-surface antigen that is expressed by clear-cell renal cell carcinoma but not detected in normal kidney or (generally) in non-clear-cell renal cell carcinoma or cancers of other organs. When ^{131}I-labeled G250 was injected intravenously 7 to 8 d before surgery, imaged lesions in the peritoneal cavity, one as small as 8 mm in diameter, were confirmed to be renal cell carcinoma. Future applications of this technology are under investigation.

5. STAGING CLASSIFICATIONS

All current staging classifications for renal cell carcinoma are based on surgical staging, i.e., after radical nephrectomy. In the United States, most classifications originated with the system reported by Flocks and Kadesky in 1958 *(70)*. Today, the most popular system in the United States is still the one proposed by Robson, Churchill, and Anderson in 1969 (Table 1) *(71)*.

The TNM (tumor, nodes, metastases) system, proposed by the International Union Against Cancer (UICC) and the American Joint Committee on Cancer (AJCC) and already commonly used in Europe, is gaining in popularity in the United States. While the TNM system is more cumbersome, it is much more precise, permitting accurate comparison of data (results) and providing the framework for a reliable estimation of prognosis. The TNM stages are based on physical examination and imaging; pathologic stages are indicated with the notations pT, pN, and pM. One problem with the TNM classification is that it has undergone periodic revision, and some tumors may be classified as one stage in one version of the system and in a different stage in another version. For example, when the 1992 TNM system was revised in 1997, T1 tumors changed from "confined to renal capsule ≤2.5 cm" to "confined to renal capsule ≤7.0 cm" *(72,73)*. Also, the N1, N2, and N3 categories of the 1987 classification were changed in 1997 to N1 and N2. The TNM stages are sometimes put into "Stage Groupings" that are designated by Roman numerals I through IV (which is potentially confusing if it is not made clear that

Table 2
TNM Classification of Renal Cell Carcinoma, 1997 Version

T— Primary Tumor
- TX Primary tumor cannot be assessed.
- T0 No evidence of primary tumor.
- T1 Tumor ≤7.0 cm in greatest dimension, limited to the kidney.
- T2 Tumor >7.0 cm in greatest dimension, limited to the kidney.
- T3 Tumor extends into major veins or invades adrenal gland or perinephric tissues but not beyond Gerota's fascia.
  - T3a Tumor invades adrenal or perinephric tissues but not beyond Gerota's fascia.
  - T3b Tumor grossly extends into renal vein(s) or inferior vena cava below diaphragm.
  - T3c Tumor grossly extends into vena cava above diaphragm.
- T4 Tumor invades beyond Gerota's fascia.

N— Regional Lymph Nodes (hilar, paraaortic, and paracaval nodes; laterality does not affect the N stage)
- NX Regional lymph nodes cannot be assessed.
- N0 No regional lymph node metastases.
- N1 Metastasis in a single regional lymph node.
- N2 Metastasis in more than one regional lymph node.

M— Distant Metastasis
- MX Distant metastasis cannot be assessed.
- M0 No distant metastasis.
- M1 Distant metastasis.

Stage Grouping			
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T1	N1	M0
	T2	N1	M0
	T3	N0, N1	M0
Stage IV	T4	N0, N1	M0
	T (any)	N2	M0
	T (any)	N (any)	M1

these are not Robson stages). A summary of the 1997 revision of the TNM classification (Table 2) is listed below:

- T1 ≤7.0 cm; limited to kidney.
- T2 >7.0 cm; limited to the kidney.
- T3 Into major veins; perinephric or adrenal invasion.
- T4 Invades beyond Gerota's fascia.
- N1 Single lymph node metastasis.
- N2 More than one lymph node metastasis.
- M0 No distant metastasis.
- M1 Distant metastasis.

Staging classifications should ideally stratify patients according to increased risk for disease recurrence and/or disease-specific mortality. The principal limitation of the Robson staging system was the inclusion of renal vein and vena cava involvement in the same stage as lymph node positive patients (stage III). Subsequent studies have demon-

strated that involvement of the renal vein or even the inferior vena cava below the diaphragm has little influence on survival. To avoid this problem, clinicians and investigators have been encouraged to use the TNM system. This system as revised in 1992 placed the cutoff between T1 and T2 tumors at 2.5 cm *(72)*. The advantage of such a cutoff is that it is extremely rare for patients with a tumor less than 2.5 cm to develop a recurrence. Unfortunately, relatively few patients actually fall into this category. On the basis of a number of reports that evaluated the optimal size cutoff, the 1997 modification of the TNM system changed the cutoff value to 7.0 cm *(73,74)*. Recently, Gettman et al. *(75)* and Bryant et al. *(76)* reported that although this cutoff value did more accurately classify tumor stage and reflect renal cell carcinoma outcome, this reclassification did not appear to affect prognosis.

Investigators at a recent UICC- and AJCC-sponsored meeting on "Diagnosis and Prognosis of Renal Cell Carcinoma: 1997 Workshop" in Rochester, Minnesota proposed the creation of subcategories of T1 for 0 to 4 cm (T1a) and >4 to 7 cm (T1b) to reflect the recommended cutoff value of 4 cm for tumors potentially manageable by nephron-sparing surgery *(77)*. The participants at this workshop also suggested that several other changes be incorporated into the next TNM revision. They proposed that T3a should include invasion of the renal sinus or of perinephric fat and that this stage include adrenal involvement only when it is secondary to direct local extension and not metastasis. Also, they recommended that macroscopic invasion of the venous wall be noted in stage T3c.

6. SUMMARY

The diagnosis and staging evaluation of renal cell carcinoma is primarily radiographic. Biopsy is rarely needed and should be only for very select patients. Current staging classifications are all based on surgical staging after nephrectomy. Of these classifications, the TNM system is the most precise and permits the best estimate of prognosis and most accurate comparison of results.

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8 Paraneoplastic Syndromes in Renal Cell Carcinoma

Riyadh Aldaabil and David Peereboom

CONTENTS

1. INTRODUCTION

Some symptoms and signs produced by renal cell cancer (RCC) such as back pain and hematuria result from a direct invasion of tissues by the tumor or its metastases. Others result from the production of biologically active substances by the tumor or by normal tissues in response to the tumor *(1–7)*. These clinical syndromes termed paraneoplastic are very important to recognize as they may be the first presenting sign of the tumor. Because these syndromes may be produced by early, localized, and potentially curable disease, it is vital not to confuse them with metastatic spread. They should also be distinguished from the toxicity of cancer therapy. Approximately 10–40% of patients with renal cancer will develop a paraneoplastic syndrome during the course of their disease *(8–9)*. A variety of signs and symptoms have been reported as paraneoplastic features of renal cancer (Table 1). Successful treatment of the tumor often leads to improvement or resolution of the paraneoplastic features.

2. SYNDROMES WITH ESTABLISHED HUMORAL FACTORS

2.1. Hypercalcemia

Hypercalcemia occurs in up to 10–20% of RCC *(4,10)* and the incidence seems to parallel the stage of disease *(11)*.

Humoral hypercalcemia of malignancy (HHM) was recognized early in the 20th century *(12)* and Allbright predicted the presence of a substance with parathyroid hormone-like activity in 1941 *(13)*. In 1964, Goldberg isolated a substance that reacted like parathyroid

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Table 1
Paraneoplastic Syndromes Associated with Renal Cell Carcinoma

Syndromes with established humoral factors
Hypercalcemia
Erythrocytosis/increased erythropoeitin
Hypertension/hyperreninemia
Increased gonadotropins
Increased prolactin
Increased enteroglucagon
Impaired glucose metabolism
Syndromes with suspected humoral factors
Nonmetastatic hepatic dysfunction
Anorexia and cachexia
Fever
Hematological syndromes
Neurological syndromes
Amyloidosis
Others

hormone (PTH) to immunoassay from a primary renal tumor and a related pulmonary metastasis in a woman with no bony metastases *(14)*. Parathyroid hormone-related peptide (PTHrP) as it was eventually called was isolated and purified from several cancers including renal cell *(15–17)*. The encoding gene was cloned and three isoforms of PTHrP with multiple PTHrP messenger RNA species were identified *(18–19)*. PTHrP shares eight of the first 16 amino acids *(18)* as well as its binding site (PTH/PTHrP receptor) *(20–21)* with PTH. Although initially thought to be tumor specific, PTHrP has been detected in normal tissue *(22–23)*. Because it is overexpressed in some malignant conditions, it may have some physiologic role apart from calcium metabolism.

2.1.1. Pathophysiology

The PTH-like biological activity of PTHrP resides in the first 34 amino acids *(24–25)*. PTHrP mediates its activity mainly by binding to the PTH–PTHrP receptor. It causes hypercalcemia by increasing bone resorption through increased osteoclastic activity, and by increasing renal tubular absorption of calcium *(26–27)*. Despite sharing a common receptor, PTH and PTHrP produce distinct syndromes (Table 2) suggesting a possible role for other factors. Tumor necrosis factor *(28)*, interleukin-6 (IL-6) *(29)*, and interleukin-1α (IL-1α) *(30–31)* are some of the many factors investigated for which exact roles remain to be elucidated. This possible interaction with other factors is further supported by the lack of correlation between the serum concentration of PTHrP and calcium *(10)*.

The presence or absence of bony metastases is probably not valid for classifying hypercalcemia associated with malignancy into humoral (paraneoplastic) or direct osteolytic (with bony metastases) as PTHrP may be elevated and probably plays a role in a subset of patients with direct bone involvement by tumor *(24,27)*.

Although PTHrP is the main cause of humoral hypercalcemia of malignancy (HHM), tumors can occasionally produce PTH *(32–33)*. Occasional patients may also have concomitant primary hyperparathyroidism *(34–35)* (Table 2).

Table 2
Differences Between Humoral Hypercalcemia of Malignancy and Primary Hyperparathyroidism

	HHM	*Primary Hyperparathyroidism*
Duration	Short	Long
Serum Ca^{++}	Increased	Increased
Serum phosphate	Decreased	Decreased
Serum PTH	Decreased[a]	Increased
Serum PTHrP	Increased	Detectable
Cyclic AMP	Increased	Increased
1,25 D3	Decreased	Increased
Pancreatitis	Not increased	Increased
Urolithiasis	Not increased	Increased

[a]Except occasional true elevation of PTH or concomitant primary hyperparathyroidism.

Key: Ca^{++}, calcium; PTH, parathyroid hormone; PTHrP, parathyroid hormone-related peptide; AMP, adenosine monophosphate; 1,25D3, 1,25dihydroxyvitaminD.

2.1.2. Clinical Features

The most common clinical features of hypercalcemia are fatigue, anorexia, nausea, vomiting, constipation, polyuria, polydipsia, and volume depletion. Cardiac signs include a range of atrial and ventricular arrhythmia, and the classic shortening of the QT interval. Neurological symptoms include lethargy and confusion, eventually leading to coma, seizures, and death *(36–37)*.

2.1.3. Treatment

As with most paraneoplastic syndromes, the most effective treatment of HHM is the successful treatment of the underlying malignancy *(37)*. However, well-established treatment modalities are available to control the calcium concentration and thus ameliorate the symptoms. The first step in treating hypercalcemia is hydration to restore intravascular volume and to increase renal clearance of calcium *(37–38)*. The latter can be further enhanced by the use of loop diuretics *(39)*. The introduction of bisphosphonates, which directly inhibit osteoclast activity, has revolutionized the drug therapy of hypercalcemia. Their effectiveness, ease of use, and acceptable side effect profile have made bisphosphonates the mainstay of drug therapy of hypercalcemia *(40)*. Pamidronate is probably the most effective bisphosphonate *(40–41)*. Other drugs such as corticosteroids, calcitonin, gallium nitrate and mithramycin have been used with variable results and side effect profiles *(36)*. These drugs are generally considered second line drugs for hypercalcemia.

2.1.4. Prognosis

Hypercalcemia is usually associated with poor prognosis and limited life expectancy *(11,36,42–43)*. However, the significance of the level of calcium is unclear *(44)*.

2.2. Erythrocytosis

Although increased erythropoeitin occurs in up to two thirds of patients with RCC *(45)*, erythrocytosis is much less common *(2–3,46–47)*. More commonly, patients with RCC have anemia that must be considered when evaluating levels of erythropoeitin.

Gross *(48)* found that 48 of 49 patients with RCC and elevated levels of erythropoeitin were anemic. The direct production of biologically active erythropoeitin or erythropoeitin-like substance by tumor cells has been established in humans as well as mice with implanted RCC cell lines *(49–52)*. However a consistent correlation between elevated erythropoeitin levels and erythrocytosis has not been established *(47)*. These results suggest that multiple factors affect erythropoeisis in the patient with RCC. Because some researchers suggested that production of erythropoeitin by tumor cells might be a physiological response to oxygen-starved cells in the center of tumor, Schiramizu et al. *(52)* have shown that erythropoeitin secretion was constitutive and was not induced by hypoxia.

Erythrocytosis, secondary to tumors, is rarely high enough to require treatment, although, phlebotomy could be employed for hematocrit more than 55% *(1)*. Normal erythropoeitin (and hematocrit) levels are usually restored after resection of the tumor *(4,53–54)*. Some researchers have suggested that elevated erythropoeitin level confers a poor prognosis and correlates with a higher stage of disease *(47)*.

2.3. Hypertension

About 25–40% of patients with RCC have hypertension *(3–4)* and the incidence of RCC in patients evaluated for hypertension is almost 10-fold the expected frequency *(55)* (*see* Chapter 1). In 1975 Hollifield *(56)* reported the discovery of RCC in a 29-year-old woman with uncontrolled hypertension. Blood pressure normalized immediately after nephrectomy. No evidence of renal artery stenosis was found and high level of serum renin was lateralized to the side of the tumor. The tumor content of renin was 40 times the level in surrounding tissue.

Elevated levels of plasma renin occur in more than one third of patients with RCC *(45, 56)* although a definite correlation with hypertension has not been found. Some patients, however, experience a reduction in plasma renin with normalization of blood pressure following nephrectomy.

Hypertension in the setting of RCC can also be caused by other factors such as A–V fistulas, polycythemia, ureteral obstruction, or hypercalcemia *(3)*.

2.4. Gonadotropin

Gonadotropin production by RCC is uncommon. Beta-human chorionic gonadotropin (β-HCG) production occurs in up to 6% of patients *(3)*. In men, excess β-HCG may cause gynecomastia, testicular atrophy, and loss of libido. High levels of estradiol may result from enhanced Leydig cell production of testosterone, which is converted to estrogen *(57–58)*. Golde described a man with RCC and elevated levels of β-HCG, placental lactogen, and placental alkaline phosphatase *(59)*. Generally, nephrectomy has been associated with a decline in serum β-HCG *(58)*. In women virilization and amenorrhea with elevated androgen levels has been described *(60)*.

2.5. Prolactinemia

Two cases of ectopic prolactin production in association with RCC have been reported. Turkington *(61)* described a 49-year-old woman who presented with fever, galactorrhea, and hematuria. Her prolactin level was high, but normalized several days after a nephrectomy. Tissue culture and antiprolactin antibodies documented prolactin production from tumor cells. Stanisic *(62)* reported on a 42-year-old man with RCC, gynecomastia, and elevated prolactin level, which also normalized after nephrectomy.

2.6. Cushing's Syndrome

In a review of 232 cases of Cushing's syndrome, Riggs and Sprague reported three cases with associated RCC *(63)*. One patient, however, developed the syndrome years after the diagnosis of the tumor and another had adrenal hyperplasia. In the third case, the syndrome resolved after nephrectomy, but a partial adrenalectomy was performed simultaneously. It remains unclear whether RCC is associated with Cushing's syndrome.

2.7. Enteroglucagon

Enteroglucagon, which is usually produced by intestinal mucosa, was markedly elevated in a patient with RCC who presented bowel malabsorption and a bowel motility disorder *(64)*. Resolution of symptoms and normalization of enteroglucagon level occurred after nephrectomy. Extracts of the resected kidney contained high levels of enteroglucagon *(65)*.

2.8. Impaired Glucose Metabolism

Four reports of abnormal glucose metabolism in association with RCC were described in *(66–69)*. Three patients presented with uncontrolled hyperglycemia despite insulin administration. Hyperglycemia promptly resolved after nephrectomy although no definite endocrine factor was isolated *(66–68)*. In the fourth patient, elevated levels of both insulin and glucagon were detected and the blood sugar fluctuated between hyperglycemia and hypoglycemia in relation to the insulin/glucagon ratio *(69)*. Blood sugar normalized after nephrectomy, but slightly elevated levels of both hormones persisted possibly because of documented metastases *(69)*.

3. SYNDROMES WITH SUSPECTED HUMORAL FACTORS

3.1. Nonmetastatic Hepatic Dysfunction

In 1961 Stauffer described a syndrome of increased alkaline phosphatase, thymol turbidity, prolonged prothrombin time (PT), increased α-globulins, and hepatosplenomegaly in the absence of liver metastases in patients with RCC *(70)*. Many more reports followed *(71–75)*. Because the absence of liver metastases is paramount to the diagnosis, other features including biochemical markers (increased alkaline phosphatase, increased transaminases, prolonged PT, increased α-2 globulin, decreased albumin) and hepatosplenomegaly occur with varying frequency. Fever, weight loss, and fatigue have been considered part of the syndrome *(4,71,74)* and while common in the absence of hepatic dysfunction, they are much more common in the presence of Stauffer's syndrome *(75)*.

The pathophysiology of Stauffer's syndrome is unclear. In most patients the syndrome resolves after nephrectomy and may recur with recurrence of the cancer *(70–71,74–75)* suggesting involvement of a humoral substance. Examination of liver biopsy specimens from these patients reveals nonspecific hepatitis with abundance of kupfer cells *(71,74)*. Although it is not clear if hepatic dysfunction has any prognostic significance, patients in whom the syndrome was not reversible or in whom it recurred had a much shorter survival *(71)*. Some researchers also suggested an association with high-grade lesion and more advanced disease *(74)*.

3.2. Anorexia and Cachexia

The syndrome of anorexia, wasting, weight loss, fatigue and poor performance status is not unique to RCC as it can occur in almost all cancers *(76)*. Anorexia and weight loss

have a significant impact on the survival of patients with cancer and can limit their ability to tolerate treatment *(1,77–78)*.

The pathophysiology of anorexia and cachexia is multifactorial. Decreased oral intake of food can be mechanical in nature, but tumor-related substances might affect the patient's taste and perception of food *(1,76)*. The role of neurotransmitters such as serotonin and neuropeptide Y in appetite regulation is still unclear *(1,78)*.

Although tumors can consume significant calories, competition with normal tissues for nutrients does not entirely explain cachexia in cancer patients. Because the theory of a hypermetabolic state is supported by abrogation of high-energy expenditure after tumor resection, it remains controversial *(79–81)*.

All aspects of metabolism seem to be affected in cachectic cancer patients. Glucose intolerance similar to that in diabetic patients has been documented *(1,82)*. In addition, these patients have accelerated gluconeogenesis with breakdown of protein and fat. This excessive consumption of tissues is not slowed by the administration of nutrients *(78)*.

Several cytokines such as tumor necrosis factor alpha (TNF-α, cachectin), interferon-gamma (INF-γ), interleukin-1 (IL-1), and interleukin-6 (IL-6) have been implicated in tumor cachexia *(1)*. These cytokines are likely produced by the host in response to the tumor, rather than by the tumor itself *(1)*.

Treatment of patients with cancer cachexia should concentrate on adequate enteral caloric intake. Parenteral nutrition has limited or no value *(83)* and it should be confined to temporary use in patients with contraindication to enteral feeding. While many medications have been tried to combat cachexia, most have limited efficacy. Cyproheptadine and hydrazine have provided no benefit in most studies *(84,85)*. Corticosteroids, while potentially helpful, can be used only for short periods without the risk for significant side effects *(78)*. Cannabinoids, such as dronabinol, extensively studied in AIDS cachexia, have shown mixed results in cancer patients *(86)*. Megestrol acetate has been shown in prospective randomized trials to improve appetite with few side effects *(87)*. Anticytokines such as TNF-α inhibitor, thalidomide, may find a niche in the treatment of cancer cachexia *(88)*.

3.3. Fever

Fever occurs in 20–40% of patients with RCC *(3–4.9)*, and it is a presenting sign in 2–12% *(2)*. Several cytokines including TNF, interferon (INF), IL-1, and IL-6 have been implicated in "tumor fever" *(89)*. Although cytokines are secreted mainly by leukocytes, their production has been confirmed in RCC cell lines *(89–91)*. These cytokines cause fever by raising the hypothalamic set point for thermal regulation *(89)*.

Management of fever in association with RCC should focus on ruling out infections. Nephrectomy as a definitive treatment can abolish fever, although a relapse can cause a return of febrile episodes *(3)*. NSAIDS remain the best pharmacological treatment for fever in association with RCC *(89,92)*. Some researchers advocate use of positive response to NSAIDS to differentiate tumor fever from that caused by infection *(93)*.

3.4. Hematological Syndromes

3.4.1. Anemia

Anemia is a more frequent in RCC than the better-known erythrocytosis. Because anemia in cancer patients can have many causes (e.g., iron deficiency, bleeding, marrow invasion by the tumor), these should be differentiated from paraneoplastic anemia. This typically normocytic normochromic anemia *(94)* is a form of anemia of chronic disease

(anemia of inflammation). The release of cytokines such as TNF-α and IL-2 might blunt the physiological response of erythropoeitin and can impair the reutilization of iron *(94,95)*. Autoimmune hemolytic anemia also occurs in the setting of RCC *(96)*, but is probably uncommon.

Treatment of RCC with surgery and/or chemotherapy may transiently worsen the anemia before improvement. Although recombinant erythropoeitin reduces transfusion requirements and improves quality of life in general *(94)*, its role in RCC has not been specifically studied. Blood transfusions remain the most common treatment for symptomatic anemia.

3.4.2. Coagulation Factors

Sufrin et al. *(97)* reported elevated levels of fibrinogen and fibrin degradation products (FDP) in a group of 30 patients with RCC. The degree of elevation correlated with the stage of disease. Prothrombin time was normal and fibrinogen levels dropped after nephrectomy. Dawson et al. *(98)* reported on a 61-year-old woman with RCC who had a prolonged prothrombin time and partial thromboplastin time with abnormal thrombin and reptilase time and elevated fibrinogen level. These parameters returned to normal after nephrectomy, but recurred when the patient developed lung metastases.

3.4.3. Thrombocytosis

Thrombocytosis in patients with RCC is thought to be secondary to IL-6 *(99)*.

3.5. Neurological Syndromes

A variety of neurological syndromes have been reported in association with RCC. These include bilateral diaphragmatic paralysis *(100)*, sensorimotor neuropathy *(101)*, motor neuron disease mimicking amyotrophic lateral sclerosis *(102)*, opsoclonus *(103)*, limbic encephalitis *(104)*, myopathy *(105)*, and polymyositis *(106)*. The neurological findings may precede the diagnosis of cancer *(103)*. Signs and symptoms usually improve or resolve after nephrectomy *(102–106)* although they may return with recurrence of the tumor *(106)*.

3.6. Amyloidosis

Amyloidosis occurs in 3–5% of RCC patients *(8)* which is second after lymphoid malignancies as the most common cause of cancer-associated amyloidosis. The protein in the amyloid fibrils is thought to be similar to that found in chronic inflammation (AA protein) *(107)*. The significance, if any, of reactive plasmacytosis occasionally found in RCC patients is unknown *(4)*.

3.7. Miscellaneous

Other "paraneoplastic" features of RCC include cutaneous lesions such as pemphigus *(108–110)* and vasculitis *(111,112)*. The latter may result from host immune sensitization by tumor antigens *(111)*.

4. CONCLUSION

Paraneoplastic syndromes are common features of RCC. Most paraneoplastic syndromes appear to be mediated by humoral factors although the pathophysiology has been defined for only a minority of these syndromes. Successful treatment of the underlying malignancy will reverse these syndromes in the majority of patients. However, even in metastatic RCC these syndromes can be controlled to improve the quality of the patient's life.

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9

Prognostic Factors in Metastatic Renal Cell Carcinoma

Paul J. Elson

CONTENTS

1. INTRODUCTION

It is estimated that almost 30,000 persons were diagnosed with renal cell carcinoma (RCC) in 1998 and that 11,600 deaths were caused by this malignancy *(1)*. If detected early, prior to metastasis, many patients can be cured surgically. The estimated 5-yr survival for patients with disease confined to the kidney (stages T_1 and T_2) is approximately 90–95% *(2)*. However, once metastatic disease develops, the prognosis for long-term survival is poor, with estimated 5-yr survival of 0–20% *(2)*. Unfortunately, approximately one-third of patients has metastatic disease on initial presentation *(2)*, and therefore, effective treatment strategies for this disease are clearly needed.

An important consideration in evaluating new treatment strategies is the role of prognostic factors. The natural history of RCC is influenced by a number of clinical and biological factors. Use of recognized prognostic factors and identification of new factors can help tailor treatment approaches to specific subgroups of patients. In addition, when comparing the relative merits of different treatments, knowledge of the factors that can influence outcome can help clarify differences between the treatments and help determine the extent to which these treatments are altering the natural history of the disease.

As discussed below, several clinical factors have been recognized for a number of years as being of prognostic value. With the advent of biologic response modifiers (BRMs), such as the interferons and interleukins, the recognition that RCC is associated with a

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number of immunological defects such as impaired intracellular signal-transduction pathways in T lymphocytes *(3)*, and the development of molecular-based technologies, a host of new potential prognostic indicators are also being identified. Although, individually, many of these factors are correlated with survival, it is important that they also be examined within the context of recognized factors. This is to ensure that the new indicators contain independent information on prognosis and that their importance is not simply because of an association with other known factors. Such analyses are being conducted and identification of new prognostic factors is helping researchers to better understand the underlying biology and natural history of RCC.

2. DEMOGRAPHIC AND CLINICAL FACTORS

Patient and disease characteristics that are easily obtained and generally part of patients' work-up have been extensively studied as potential prognostic factors. These include patient demographics, constitutional factors such as performance status (P.S.), paraneoplastic syndromes such as weight loss, elevated erythrocyte sedimentation rate (ESR), fever, and anemia; disease-related factors such as sites of metastatic disease, disease-free interval, tumor size, cell type, and histologic grade; treatment factors such as prior systemic therapy and previous nephrectomy; and laboratory tests such as serum chemistries and blood counts.

These factors have been studied both individually using univariate data analysis methods, and in groups using multivariate methods. Univariate analyses consider only a single factor, and its prognostic value is evaluated without regard to possible associations with other factors. Multivariate methods, on the other hand, permit several factors to be simultaneously assessed. This allows the impact of each factor to be examined, while taking into account the possible effects of the others. For the evaluation of prognostic factors, the univariate approach is a good way to identify candidate factors. However, as aforementioned, multivariate methods are needed to determine if a candidate is providing additional, independent information.

For completeness, studies that utilized only univariate methods, as well as those which employed both univariate and multivariate analyses are discussed below. Table 1 provides a summary of the results of published reports of patient demographic and clinical prognostic factors that utilized both univariate and multivariate data analysis methods. For each study, Table 1 gives the number of patients studied, whether the study was conducted during the chemotherapy or BRM "era," the factors evaluated, and the univariate and multivariate results. It is important to remember when interpreting these data that all of the reports are retrospective in nature and most have combined data from several clinical trials with differing inclusion criteria.

2.1. Demographics

Most studies including many of those summarized in Table 1 have found no association between survival and sex *(4–15)*, age *(4,6–9,11–16)*, or race *(7,12)*. An exception to this is an early study by Klugo *(17)* which suggested males have a better prognosis then females.

2.2. Constitutional Factors and Presenting Symptoms

P.S., which attempts to measure overall well-being, is one of the most important prognostic factors in advanced RCC identified to date and most reports have found a clear

relationship between it and survival *(4,7–13,16,18)*. Based on the reports summarized in Table 1 and regardless of the era of the studies involved, patients with excellent P.S. (ECOG 0 or Karnofsky (KPS) 100) have median survival of 10–15 mo *(7,8,12)* whereas patients with ECOG P.S. 1 (KPS 80-90) have a median survival of 6–8 mo *(7,8,12)*. Patients with poorer P.S. (ECOG ⊕ 2, KPS < 80) have a median survival of only 2–5 mo *(7,13)*. The prognostic value of P.S. is not a universal finding, however. In a study of 134 patients treated between 1971 and 1986 de Forges *(5)* found P.S. to be prognostic in univariate analysis, but not in multivariate analysis once the effects of sites of metastatic disease, weight loss, and ESR were accounted for. Similarly, Lopez-Hänninen *(19)* failed to find an association between P.S. and survival once the effects of metastatic sites, ESR, pretreatment hemoglobin, LDH, and neutrophil count were accounted for. Despite these contradictory results, and the fact that P.S. is subjective, it appears to be a powerful predictor of survival.

Recent weight loss, which can also be thought of as a measure of general health, has also been identified as an important prognostic factor *(5–7,10)*. Although often correlated with P.S., these studies found that even after adjusting for P.S., patients who experienced recent weight loss had a poorer prognosis than patients whose weight remained stable. As with P.S., the value of weight loss as an independent prognostic factor is not a universal finding, and several reports *(8,12,19)* found no association with survival. A possible explanation for this is patient selection. The reports by de Forges *(5)* and Elson *(7)*, for example, included only patients treated with chemotherapy. P.S. ranged from ECOG 0–3 and 44% and 47% of patients, respectively, experienced recent weight loss. In contrast, the reports by Palmer *(8)* and Mani *(12)* included only patients treated with BRMs. P.S. was restricted to ECOG 0 and 1 and only 11% and 15% of patients, respectively, experienced weight loss.

In addition to P.S. and weight loss, several investigators have evaluated the effects on survival of anemia and inflammatory markers such as ESR.

Anemia (hemoglobin < 10 gm/dL) as an independent prognostic factor for survival has been reported by several investigators *(13,14,19)*. These investigations have found that even after correcting for other important predictors patients with baseline hemoglobin < 10 gm/dL had a poorer prognosis than patients with hemoglobin ⊕ 10 gm/dL. Hoffmann *(15)*, on the other hand, found no association between hemoglobin levels and survival. The reason hemoglobin levels should appear to be of prognostic value is unclear. Citerrio *(13)* suggested that low hemoglobin levels might limit the ability to administer aggressive treatment, and/or that decreased hemoglobin levels might be an indication of impaired hematopoetic function and advanced disease.

Acute phase proteins are nonspecific indicators of local or systemic inflammation which are often elevated in patients with RCC. Ljungberg *(20)* studied the impact of ESR, C-reactive protein (CRP), haptoglobin, ferritin, orosomucid, and α1-antitrypsin in 170 unselected patients with RCC. All six parameters were found to be correlated with each other and, in univariate analyses with survival. ESR, however, was the only factor that was found to provide independent prognostic information in multivariate analysis. Adjusting for tumor stage and grade, patients with ESR ⊕ 54 mm/h had shorter survival than patients with ESR < 54 mm/h. Lopez-Hänninen *(19)* observed similar results in that ESR, but not CRP was found to be prognostic in a series of 215 consecutive patients treated with various IL-2 based therapies. Hoffmann *(15)*, on the other hand found both ESR and CRP to be independent prognostic factors in a series of 99 patients treated with

Table 1
Patient and Disease Factors–Results from Multivariate Analyses[a]

Reference	*N*	*Era*	*Performance Status*	*Recent Weight Loss*	*DTI or DMI in Months[b]*	*Metastatic Sites*	*ESR (mm/hr)[c]*
de Forges *(5)*	134	Chemo.	(U)	£10% vs >10%[d]	>0 vs 0	Liver: no vs yes Lung: no or <2 cm and limited to one site vs ≈2 cm and/or >5 lesions/field	<50 vs 50-99 vs ≈100[d]
Neves *(6)*	158	Chemo.	(NA)	<10% vs ≈10%	(NA)	Single site vs multiple sites	(NA)
Elson *(7)*	610	Chemo.	ECOG 0 vs 1 vs 2 vs 3	No vs Yes	>12 vs £12	No. sites: £1 vs >1 based on: lung, liver, brain, and "other"	(NA)
Palmer *(8)*	327	BRM	ECOG 0 vs 1	(NS)	>24 vs £24	No. sites: 1 vs >1 based on: lung, bone, and "other"	(NA)
Minasian *(9)*	159	BRM	KPS≈80 vs <80	(NA)	(U)	(NS)	(NA)
Fosså *(10)*	295	Chemo./BRM	ECOG 0,1 vs 2,3	£10% vs >10%	>12 vs £12	(U)	<50 vs 50-99 vs ≈100
Canobbio *(11)*	73	BRM	ECOG 0 vs 1,2	(NA)	(U)	No. sites: 1 vs >1	(NA)
Mani *(12)*	84	BRM	ECOG 0 vs 1	(U)	(NS)	Bone: No vs yes	(NA)
Fyfe *(16)*	255	BRM	ECOG P.S.[e]	(NA)	Absolute DTI	(NS)[f]	(NA)
Lopez-Hänninen *(19)*	215	BRM	(NS)[f]	(NS)[f]	(NS)[f]	Extrapulmonary vs lung only Bone: No vs yes	£70 vs >70 (NA)
Citterio *(13)*	109	BRM	ECOG 0,1 vs 2,3	(NA)	(U)	(U)	
Wittke *(14)*	80	BRM	(NA)	(NA)	(NS)	(NS)	<50 vs ≈50
Hoffman *(15)*	99	BRM	(NA)	(NA)	(NS)	(NS)	<70 vs ≈70

Reference	*Nephrectomy*	*Prior Systemic Treatment*	*Other Factors Associated with Survival*	*Factors Examined but not Associated with Survival*
de Forges *(5)*	(U)	(NA)	Fever > 38°C: No vs Yes (U)	Sex, left vs right sided tumor
Neves *(6)*	(NA)	(NA)	Grade: Low (*1,2*) vs High (*3,4*)	Sex, age, cell type, left vs right sided tumor, size of primary
Elson *(7)*	(U)	No vs Yes	None	Sex, age, race, prior radiotherapy
Palmer *(8)*	(U)	(NS)	None	Sex, age, prior radiotherapy
Minasian *(9)*	Yes vs No	(NA)	None	Sex, age
Fosså *(10)*	(U)	(NA)	Age: £60 vs >60 (U)	Sex
Canobbio *(11)*	(NS)	(NS)	None	Sex, age
Mani *(12)*	(U)	(NS)	Other histologies vs sarcomatoid	Sex, age, race, prior radiotherapy
Fyfe *(16)*	Yes vs No	(NS)	None	Age
Lopez-Hänninen *(19)*	(NS)[f]	(NS)[f]	Hgb ≈ 10 vs <10 gm/dL LDH £ 280 vs >280 U/L Neutrophils £ 6000 vs >6000/mL	Size of primary, leukocyte count, gamma GT, alkaline phosphatase, CRP
Citterio *(13)*	(NA)	(NA)	Hgb > 10 vs £10 gm/dL Grade (U) Serum albumin (U) Serum calcium (U) Serum LDH (U) Serum Alkaline Phosphatase (U)	Sex, age, serum creatinine, serum ferritin, serum triglycerides
Wittke *(14)*	(NA)	(NA)	Hgb > 10 vs £10 gm/dL LDH < 240 vs ≈240 U/L IL-10 £ 1 vs >1 pg/mL	Sex, age
Hoffman *(15)*	(NA)	(NA)	sICAM-1<360 vs ≈360 ng/mL CRP < 8 vs ≈8 mg/L Neutrophils£ 6000 vs >6000/mL (U) Serum LDH (U)	Sex, age, hemoglobin, sELAM-1, sVCAM-1

[a] Level of each factor associated with better prognosis is listed first; (U)=Statistically significant in univariate analysis, but not associated with survival in multivariate analysis; (NS) = Not statistically significant in univariate or multivariate analysis; (NA) = Not assessed

[b] DMI: Time from diagnosis to metastatic disease, DTI: Time from diagnosis to systemic treatment

[c] Erythrocyte sedimenation rate

[d] Authors used a combination of weight loss and sedimentation rate in multivariate analysis: no weight loss and ESR < 100 mm/h vs weight loss and/or ESR ≈100mm/h

[e] Includes P.S. 0-4, however, how levels are compared is not given

[f] Univariate results not reported, but not associated with survival in multivariate analysis

Table 2
Metastatic Sites[a]

Metastatic Site	*References for Studies Where the Site was Prognostic*	*References for Studies Where the Site was Not Prognostic*
Lung : No vs Yes	*7,8*	*6, 9, 10,11,12,14,15*
Lung only: No vs Yes	*4,23*	*16*
No or limited involvement vs extensive involvement	*5*	None
Lung ± lymph nodes vs other sites	*13* (U)	None
Lung ± other sites vs only extrapulmonary sites	*19*	None
Liver: No vs Yes	*5,6* (U),*7,10* (U)	*8,11,12,13–15,19*
Bone: No vs Yes	*7* (U),*8,12,19*	*5,6,9,11,13–15*
CNS: No vs Yes	*7*	*5,14,15*
Lymph Nodes: No vs Yes	None	*5,12,13,19*
Other Sites: No vs Yes	*8*	*5,6,7,13*
Single vs multiple sites	*6,7,8,11*	*9*[b]*,13,16,19*

[a] Level of the factor associated with better prognosis is listed first; (U) = Statistically significant in univariate analysis, but not associated with survival in multivariate analysis.
[b] ≤2 sites vs >2 sites.

immunotherapy with or without chemotherapy. Other investigators studying only ESR *(5,10,14,21)* that also found it to be an independent predictor of survival. In addition to acute phase reactants as indicators of an inflammatory process, de Forges *(5)* found that patients who presented with fevers >30°C had a worse prognosis than patients without fever on univariate analysis, however, the importance of fever was lost once the effects of weight loss, ESR, and sites of metastatic disease were taken into account.

2.3. Disease-Related Factors

RCC most frequently metastasizes to the lungs, liver, bone, and lymph nodes *(22)*. Sites of metastatic spread and tumor burden as measured by the number of metastatic sites involved have been extensively studied with mixed results. Table 2 summarizes these results in terms of which studies have found specific metastatic sites and/or tumor burden to be prognostic and which have not. Minasian *(9)*, Wittke *(14)*, Hoffmann *(15)*, and Fyfe *(16)* found no association between survival and metastatic sites. Most other investigators, however, have found location and/or number of metastatic sites to be of prognostic value in univariate and/or multivariate analyses. Which sites are relevant, how-ever, is unclear. With the exception of lymph nodes and "other" sites, Table 2 indicates that lung, liver, bone, and brain metastases have been found to be of prognostic value in approximately 40% of the studies in which they were evaluated and not associated with survival in the other 60%. Table 2 also suggests, however, that the specific sites of metastatic spread may not be as important as the total tumor burden. Here again, however, half of the studies that examined the number of metastatic sites involved found it to be an independent predictor of survival and the other half did not.

Metastasis-free interval (initial diagnosis to development of metastatic disease or treatment for metastatic disease) has also been extensively studied. Several investigations have not found metastasis-free interval to be of prognostic value *(12,14,15,19)*,

however, most have *(4,5,7–11,13,16,23)*. Different cutoffs were used in these studies to divide patients into prognostic subgroups. de Forges *(5)*, for example, used metastatic disease at the time of diagnosis vs anytime thereafter. Elson *(7)*, Minasian *(9)*, Fosså *(10)*, and Canobbio *(11)* used a cutoff of 1 yr, Plamer *(8)*, and Citterio *(13)* used a 2-yr cutoff, and Fyfe *(16)* considered metastasis-free interval as a continuous measure. Regardless of how metastasis-free interval was characterized, however, the results of these studies were qualitatively similar. That is, patients found to have concurrent metastatic disease at the time of initial diagnosis or shortly thereafter had a poorer prognosis than patients who developed metastatic disease well after their initial diagnosis.

In addition to the extent of metastatic disease and metastasis-free interval, several investigators have also examined the effects on survival of tumor size, location of the primary, i.e., right or left kidney, and routine histologic features, such as cell type and grade.

Location of the primary tumor does not appear to impact survival *(5,6)*, however, there is some indication that the size of the primary may. In a study of 2473 patients diagnosed with RCC between 1975 and 1985, Guinan *(24)* found a significant correlation between size of the primary tumor and survival separately in patients with stages II, III, and IV disease. Among 441 patients with stage IV disease, patients with tumors less than 5 cm in diameter had estimated 5-yr survival of 28% compared to 13% for patients with 5–7.5 cm tumors, 20% for patients with 7.6–10-cm tumors, and 10% for patients with tumors greater than 10 cm in diameter ($p = 0.02$). Neves *(6)* and Lopez-Hänninen *(19)*, however, found no association between the size of the primary and survival in multivariate analyses.

In a recent study of 84 patients treated with BRMs, Mani *(12)* reported that patients with sarcomatoid tumors had significantly worse survival than patients with other histologies. Neves *(6)*, however, found no association between cell type and survival. It should be pointed out that these results are based on relatively small numbers of patients and incomplete descriptions of the histologies studied. The report by Mani, for example, included only five patients (6%) with sarcomatoid tumors and the report by Neves did not describe the histologies included in the study.

Tumor grade has also been studied as a potential prognostic factor and Neves *(6)* found a significant correlation between tumor grade and survival in a multivariate analysis. Citterio *(13)*, on the other hand found grade not to be an important predictor of survival once P.S. and hemoglobin levels were accounted for.

2.4. Treatment-Related Factors

Prior systemic therapy has been suggested as being correlated with poor survival *(7)*, however, most reports have failed to find an association with outcome *(8,11,12,16,18,19)*. Similarly, prior radiotherapy has not been found to be of prognostic value *(7,8,12)* in this disease. Prior nephrectomy, on the other hand, has been found to be a favorable prognostic factor in two multivariate analyses *(9,16)*, and a number of univariate analyses *(4,5,7,8, 10,12)*. Its prognostic value was lost, however, once other factors were accounted for. One explanation for this is that prior nephrectomy and P.S. are highly correlated *(4,7,12)*, and, therefore the observed value of prior nephrectomy may simply be a reflection of other patient factors.

2.5. Laboratory Factors

Laboratory measures have not been studied as extensively as the clinical factors described above. Several investigators, however, have found pretreatment neutrophil count *(19)*, LDH

(14,19), serum calcium *(25)*, pseudouridine *(26)*, and γ-enolase *(27)* to be associated with survival, whereas other investigators have failed to find such associations. Hoffman *(15)*, for example, found neutrophil count and LDH to be important predictors in univariate analyses but not in multivariate analysis after correcting for other important factors. Similarly, Citterio *(13)* found LDH and serum calcium to be of no prognostic value in multivariate analysis, though they were important preditors in univariate analyses. Additional laboratory measures that have been studied, but not found to be prognostic for survival include pretreatment leukocyte count *(19)*, alkaline phosphatase *(13,19,28)*, serum creatinine *(13)*, serum albumin *(13)*, serum triglycerides *(13)*, and β_2 microglobulin *(29)*.

3. DNA PLOIDY AND NUCLEAR MORPHOMETRY

The value of DNA ploidy as a prognostic factor is controversial. Some studies have found no association between ploidy and survival *(30,31)*, whereas others have found patients with diploid tumors to have either a better *(32)* or worse *(33)* prognosis than patients with aneuploid tumors. A possible explanation for these results is tumor heterogeneity. Ljungberg *(32)*, for example, recently conducted a study in which multiple tumor samples were studied. He observed that the likelihood of finding aneuploid cell clones within a true aneuploid tumor increased in relation to the number of samples evaluated, and calculated the sensitivity of finding an aneuploid clone from a single sample to only be approximately 70%.

Because of the heterogeneity of RCC and the subjectivity associated with other histological characteristics, such as nuclear grade, a number of investigators have suggested using quantitative measures of nuclear morphology as more objective predictors of outcome. The morphologic features studied can be divided into essentially two groups: size parameters and shape parameters. The size factors include nuclear perimeter, area, major and minor axes, and mean nuclear volume. The shape parameters primarily include measures of "roundness" and irregular shape. In addition to these size and shape parameters, chromatin texture has also been studied. Many of these parameters are correlated with each other and in univariate analyses, survival *(34–38)*. The results of multivariate analyses of subsets of these parameters and clinical factors, however, have been mixed. Ruiz *(34)*, for example, studied nuclear area, perimeter, major and minor axes, and a shape factor and found none that provided independent prognostic information. van der Poel *(35)*, on the other hand, found chromatin texture, but no size or shape parameters, to be an independent predictor in 52 patients with advanced disease. Delahunt *(36)* did not study chromatin patterns, but found a nuclear "roundness" parameter to be the only morphometric parameter that provided independent prognostic information, and Artacho-Pérula *(37)* found only mean nuclear volume to be important.

Although these studies differ with respect to which parameter is most important, they do suggest that the evaluation of nuclear morphology may be an important factor in determining prognosis.

4. IMMUNOLOGICAL FACTORS

A number of immunological parameters including serum levels of interleukin (IL) 6, IL-10, and soluble IL-2 receptor (sIL-2R), and T-cell subsets have been examined in relation to their effect on survival.

4.1. Cytokine Levels and Cytokine Receptors

IL-6 is a multifunctional cytokine *(39)*. It has immunoregulatory activities and regulates the proliferation and differentiation of natural killer cells, cytotoxic T cells, and normal hematopoietic progenitors. In vitro, IL-6 has been shown to act as an autocrine growth factor for various cancer cell lines, including RCC. IL-6 also acts as an endogenous pyrogen and induces the expression of acute phase genes such as the CRP gene. Not unexpectedly, serum IL-6 levels has been shown to be correlated with ESR and acute phase proteins such as CRP *(39,40)*. Studies by Blay *(39)*, Ljungberg *(40)*, and Stadler *(41)* have demonstrated an association between elevated baseline serum IL-6 levels and poor survival. In the study by Blay, serum IL-6 levels were detected in 48% of 138 patients with metastatic RCC, and median survival was worse in these patients compared to those without detectable IL-6 (8 mo vs 16 mo median survival, $p < 0.03$). Similarly, Ljungberg found survival to be significantly better in stage IV patients with IL-6 levels < 8.3 ng/L compared to those with levels ⊕ 8.3 ng/L, $p = 0.003$, and Stadler found patients with metastatic disease and IL-6 levels ≤ 5 pg/mL to have significantly better survival than those with IL-6 levels greater than 5 pg/mL, $p = 0.04$. However, in the studies by Stadler and Ljungberg IL-6, though correlated with survival, was not an independent prognostic factor once the effects of other factors such as ESR *(40)* and number of metastatic sites *(41)* were taken into account.

IL-10, an immunosupressive cytokine which can suppress antigen presenting cells and may lead to down regulation of HLA class I and II molecules on dendritic cells, has recently been identified as an independent prognostic factor for survival by Wittke *(14)*. Correcting for hemoglobin, LDH, and ESR levels, Wittke found that patients with baseline serum IL-10 levels above 1 pg/mL had significantly shorter survival times than patients with levels of 1 pg/mL or less, $p = 0.03$. The basis for elevated IL-10 levels is unclear, however, renal carcinomas themselves have been found to produce IL-10 *(42)*.

In a small study recently conducted by Matsumoto *(43)*, serum levels of sIL-2R were found to be correlated with stage, CRP, and serum IL-6 levels, and in 17 patients with stage IV disease, survival. As with serum IL-10 levels, the basis for elevated sIL-2R is unclear. However, the source may be the tumor cells themselves or tumor activated lymphocytes *(43)*.

4.2. T-Cell Subsets

Although not extensively evaluated, several small studies have found an association between response to chemoimmunotherapy and baseline lymphocyte count, CD4+ and CD8+ T-cell counts and B-cell counts *(44)*, and between recurrence and the number of activated CD8+ T-cells postnephrectomy *(45)*. These studies involved small numbers of patients and survival was not an endpoint, however, they suggest that monitoring lymphocyte subsets may be of prognostic value.

5. MOLECULAR MARKERS

5.1. Cell Proliferation Markers

The prognostic value of several markers of cell proliferation have been studied in RCC patients including Ki-67, proliferative cell nuclear antigen (PCNA), and silver staining nucleolar organizer regions (AgNORs). PCNA and the Ki-67 antigen are expressed during the G1, S, and G2 phases of the cell cycle and, in the case of Ki-67 also during mitosis.

AgNORs are intranucleolar structures visible during interphase, which are thought to be indicative of proliferative activities. These markers have been studied both individually and in combination by a number of investigators.

Delahunt *(46)* and Tannapfel *(47)* examined all three markers in patients with stages I–IV disease. Both investigators found Ki-67 index to be an independent negative predictor of survival in multivariate analyses. Delahunt, however, also found PCNA index and AgNOR score to be independent predictors, whereas Tannapfel found both to be predictive in univariate analyses, but not in multivariate analysis after correcting for stage and Ki-67 index. Moch *(48)* also evaluated Ki-67 index, but did not find it to be correlated with survival. The study, however, was restricted to patients with pT_3 disease. Lipponen *(49)* and Cronin *(50)* studied PCNA immunostaining and both found high PCNA index to be predictive of poor survival. Yang *(51)* evaluated AgNORs and, like Tannapfel found no association with survival.

Although the results of these studies are mixed, when taken together they suggest that proliferation markers may be of prognostic value. It remains unclear, however, which marker or combination of markers will be the most useful.

5.2. Apoptosis-Related Markers

p53 is a tumor suppressor gene that acts as a negative regulator of the cell cycle. Mutations in the gene lead to production of proteins, which have a prolonged half-life and accumulate in cells to levels that are immunohistochemically detectable. Other mechanisms, such as binding to other cellular proteins, may also lead to p53 protein stabilization *(48)*. Studies of the prognostic value of *p53* have had mixed results. Moch *(48)*, for example, found overexpression of *p53* to be correlated with tumor grade, Ki-67 index, and overexpression of the *mdm-2* gene, but not metastasis status in 50 patients with pT_3 RCC. In multivariate analysis, *p53* and metastasis status, but not Ki-67, were found to be independent predictors of poor survival. Similarly, Shiina *(52)* found *p53* expression to be predictive of survival in both univariate and multivariate analyses of 72 RCC patients. As in the study by Moch, *p53* expression was correlated with grade but neither M-stage nor T-stage. A study by Uhlman *(53)* also had similar results in that *p53* expression was predictive of poor survival in patients without metastatic disease ($n = 119$, $p < 0.003$). It was not, however, associated with survival in patients with distant metastases present ($n = 45$, $p = 0.43$). Uhlman also found *p53* expression to be correlated with grade. However, contrary to the studies by Moch and Shiina, *p53* expression was also correlated with Robson stage. In contrast to these studies, Bot *(54)* and Lipponen *(49)* found no association between survival and *p53* expression.

The retinoblastoma gene (*Rb*) is also a tumor suppressor gene. Its prognostic value in RCC, however, has not be studied as extensively as *p53*. The *bcl-2* and *c-Myc* genes help regulate cell growth and mutations in these genes can inhibit apoptosis and lead to cell proliferation. Lipponen *(55)* studied the prognostic value of these three genes in 104 RCC patients, 26 of whom had metastatic disease. None of the genes was found to be an independent predictor of survival in multivariate analysis after taking into account T-stage, nuclear grade, and mitotic index.

5.3. Growth Factors

Epidermal growth factor receptor (EGF-R) is a transmembrane receptor for several ligands including EGF and transforming growth factor alpha (TGF-α). Several studies

have evaluated the prognostic value of these growth factors, but have reported mixed results. A study of 164 patients with RCC by Uhlman *(56)* found EGF-R positivity (intermediate or strong cell membrane immunostaining) to be associated with high-grade tumors and poor survival, whereas TGF-α expression was not associated with outcome. Moch *(57)*, on the other hand, found EGF-R positivity to be correlated with Ki-67 index, but not survival. These investigations studied different patient populations and used different definitions of EGF-R positivity. Consequently, it is difficult to assess the true impact of EGF-R and additional studies are needed.

*c-erb*B-2 (*HER-2/neu*) is an oncogene that encodes a protein that has structural similarities to EGF-R. A recent study of soluble extracellular *c-erb*B-2 protein by Rasmuson *(58)* found serum levels to be inversely correlated with stage and grade. In separate analyses of stage I, II–III, and IV patients, Rasmuson found elevated serum levels to have a negative impact on survival among patients with stage I disease ($n = 71$, $p = 0.05$) but no impact on survival among patients with stages II–III disease ($n = 50$) or stage IV disease ($n = 63$). Similarly, Lipponen *(49)* found no association between *c-erb*B-2 overexpression and survival in a study of tumor samples from 123 RCC patients.

5.4. Adhesion Molecules

Recent studies of intercellular adhesion molecules suggest that serum levels of soluble forms of the molecules and/or expression of membrane bound molecules may be of prognostic value. Hoffman *(15)*, for example, studied serum levels of soluble intercellular adhesion molecule-1 (sICAM-1), vascular adhesion molecule-1 (sVCAM-1), and E-selectin (sELAM-1) in 99 patients with metastatic disease. Adjusting for the effects of ESR and CRP, patients with sICAM-1 levels below 360 ng/mL had significantly better survival than patients with higher levels, $p = 0.001$. Neither sVCAM-1 or sELAM-1 was seen to have prognostic value in this study. Katagiri *(59)* studied E-cadherin expression in 106 tumor samples and found that loss of expression was associated with advanced stage disease and poor survival. Similarly, in a small study of 23 tumor samples, Anastassiou *(60)* found low expression of platelet endothelial cell adhesion molecule-1 (PECAM-1) to also be associated with decreased survival.

6. CYTOGENETICS

Recent studies of genetic aberrations in RCC suggest that the number of accumulated chromosomal aberrations and/or the presence of specific aberrations may be of prognostic value. Moch *(61)*, for example, studied 41 patients with nonmetastatic RCC and reported that loss of chromosome 9p and the total number of DNA losses per tumor were associated with a poor prognosis. Similarly, Elfving *(62)*, reported that among 50 consecutive RCC patients, those with fewer than six chromosomal alterations (and additions or deletions) had a significantly better prognosis than patients with six or more aberrations. A study of 30 patients with nonpapillary RCC by Wu *(63)* found deletion of chromosome 14q to be associated with a poor prognosis.

These studies were carried out in relatively small series of patients, few of whom had metastatic disease, and did not include survival as a primary endpoint. Despite this, these early investigations suggest that studying structural alternations of the chromosomes of patients with RCC may provide important information regarding tumor biology and prognosis.

7. CONCLUSIONS

Performance status is the most important prognostic factor for survival in patients with metastatic RCC identified to date. Although subjective, it has been shown to have excellent discriminatory power in numerous reports. Other clinical factors such as ESR, metastasis free interval, recent weight loss, and possibly sites of metastatic disease/tumor burden also appear to be important predictors. In addition to these "classical" prognostic factors, a number of immunological, molecular, and genetic factors have also been shown to correlate with survival in recent years. Their value as independent prognostic factors, however, needs to be confirmed by examining them in conjunction with the classical predictors.

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II Management of Localized Renal Cell Carcinoma

10 Radical Nephrectomy and Nephron-Sparing Surgery for Localized Renal Cell Carcinoma

Andrew C. Novick

CONTENTS

1. INTRODUCTION

Notwithstanding recent advances in our understanding of the genetics and biology of renal cell carcinoma (RCC), surgery remains the mainstay of curative treatment for this disease. Nevertheless, the role of traditional radical surgery is changing and nephron-sparing surgery (NSS) has assumed an increasing role in the management of localized tumors. These newer concepts regarding treatment of localized RCC are the primary focus of this chapter.

2. RADICAL NEPHRECTOMY

Robson et al. established radical nephrectomy as the gold standard curative operation for localized RCC with their report of 66% and 64% overall survival for stages I and II tumors, respectively *(1)*. These results demonstrated improved survival rates compared with patients treated with pericapsular nephrectomy. More recent reports indicate 5-yr survival rates of 80% or more following radical nephrectomy for stage I (T1-2) RCC *(2)*. Radical nephrectomy currently remains the established form of treatment for patients with localized unilateral RCC and a normal contralateral kidney.

The concept of radical nephrectomy encompasses the basic principles of early ligation of the renal artery and vein, removal of the kidney outside Gerota's fascia, removal of the ipsilateral adrenal gland, and performance of a complete regional lymphadenectomy from the crus of the diaphragm to the aortic bifurcation. In recent years, controversy has arisen concerning the need for some of these practices in all patients. Performance of a

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Table 1
Results of Nephron-Sparing Surgery for Renal Cell Carcinoma

Study	*No. Patients*	*Local Tumor Recurrence (%)*	*5-Yr Cancer-Specific Survival*
Morgan and Zincke *(6)*	104	6 (5.8%)	89%
Steinbach et al. *(7)*	121	5 (4.1%)	90%
Licht et al. *(8)*	216	9 (4.2%)	87%

perifascial nephrectomy is of undoubted importance in preventing postoperative local tumor recurrence because approximately 25% of localized RCCs will manifest perinephric fat involvement. Preliminary renal arterial ligation remains an accepted practice, however, in large tumors with abundant collateral vascular supply, it is not always possible to achieve complete preliminary control of the arterial circulation. It has now been well demonstrated that removal of the ipsilateral adrenal gland is not routinely necessary unless the adjacent upper portion of the kidney is involved with RCC *(3)*. Finally, the need for performance of a complete regional lymphadenectomy in all cases is unproven. Although this allows more accurate staging of the extent of RCC, the therapeutic value of this information is limited because there is no established form of systemic treatment for patients with advanced disease. The therapeutic merits of lymphadenectomy itself have not been conclusively shown, although recent data from Giuliani et al. suggest that a subset of patients with micrometastatic lymph node involvement can be benefitted *(4)*. At present, the need for routine performance of a complete lymphadenectomy in all cases is unresolved and there remains a divergence of clinical practice among urologists with respect to this aspect of radical nephrectomy.

3. NEPHRON-SPARING SURGERY

Nephron-sparing surgery (NSS) has become a successful form of treatment for patients with localized RCC when there is a need to preserve functioning renal parenchyma *(5)*. This need is present in patients with bilateral RCC, RCC involving a solitary functioning kidney, chronic renal failure, or unilateral RCC and a functioning opposite kidney that is at risk for future impairment from an intercurrent disorder. The technical success rate with NSS is excellent, and long-term patient survival, free of cancer, is comparable to that obtained after radical nephrectomy, particularly for low-stage RCC (Table 1). The major disadvantage of NSS for RCC is the risk of postoperative local tumor recurrence in the operated kidney, which has occurred in 4–6% of patients *(6–8)*. These local recurrences are most likely a manifestation of undetected microscopic multifocal RCC in the remnant kidney. The risk of local tumor recurrence after radical nephrectomy has not been studied, but it is presumably very low.

We recently reviewed the results of NSS for treatment of RCC in 500 patients managed at The Cleveland Clinic prior to December 1996. A technically successful operation with preservation of function in the treated kidney was achieved in 489 patients (98%). The mean postoperative serum-creatinine level in these patients was 1.8 mg/dL. The overall and cancer-specific 5-yr patient survival rate in the series was 81% and 93%, respectively. Recurrent RCC developed postoperatively in 38 of 473 patients (8.2%) with sporadic RCC. Thirteen of these patients (2.7%) developed local recurrence in the remnant kidney,

whereas 26 patients developed metastatic disease. These data confirm the NSS provides effective therapy for patients with localized RCC when preservation of renal function is a relevant clinical consideration.

3.1. General Operative Considerations

Evaluation of patients with RCC for partial nephrectomy should include preoperative testing to rule out locally extensive or metastatic disease. For most patients, preoperative renal arteriography to delineate the intrarenal vasculature aids in excising the tumor with minimal blood loss and damage to adjacent normal parenchyma. This test can be deferred in patients with small peripheral tumors. Selective renal venography is performed in patients with large or centrally located tumors to evaluate for intrarenal venous thrombosis secondary to malignancy. The latter, if present, implies a more-advanced local-tumor stage and also increases the technical complexity of tumor excision.

It is usually possible to perform partial nephrectomy for malignancy *in situ* by using an operative approach that optimizes exposure of the kidney and by combining meticulous surgical technique with an understanding of the renal vascular anatomy in relation to the tumor. We employ an extraperitoneal flank incision through the bed of the eleventh or twelfth rib for almost all of these operations; we occasionally use a thoracoabdominal incision for very large tumors involving the upper portion of the kidney. These incisions allow the surgeon to operate on the mobilized kidney almost at skin level and provide excellent exposure of the peripheral renal vessels. With an anterior subcostal transperitoneal incision, the kidney is invariably located in the depth of the wound, and the surgical exposure is simply not as good.

When performing *in situ* partial nephrectomy for malignancy, the kidney is mobilized within Gerota's fascia while leaving intact the perirenal fat around the tumor. For small peripheral renal tumors, it may not be necessary to control the renal artery. In most cases, however, partial nephrectomy is most effectively performed after temporary renal arterial occlusion. This measure not only limits intraoperative bleeding but, by reducing renal tissue turgor, also improves access to intrarenal structures. In most cases, we believe that it is important to leave the renal vein patent throughout the operation. This measure decreases intraoperative renal ischemia and, by allowing venous backbleeding, facilitates hemostasis by enabling identification of small transected renal veins. In patients with centrally located tumors, it is helpful to occlude the renal vein temporarily to minimize intraoperative bleeding from transected major venous branches.

When the renal circulation is temporarily interrupted, *in situ* renal hypothermia is used to protect against postischemic renal injury. Surface cooling of the kidney with ice slush allows up to 3 h of safe ischemia without permanent renal injury. An important caveat with this method is to keep the entire kidney covered with ice slush for 10–15 min immediately after occluding the renal artery and before commencing the partial nephrectomy. This amount of time is needed to obtain core renal cooling to a temperature (approx 20°C) that optimizes *in situ* renal preservation. During excision of the tumor, invariably large portions of the kidney are no longer covered with ice slush and, in the absence of adequate prior renal cooling, rapid rewarming and ischemic renal injury can occur. Cooling by perfusion of the kidney with a cold solution instilled via the renal artery is not recommended because of the theoretical risk of tumor dissemination. Mannitol is given intravenously 5–10 min before temporary renal arterial occlusion. Systemic or regional anticoagulation to prevent intrarenal vascular thrombosis is not necessary.

A variety of surgical techniques are available for performing partial nephrectomy in patients with malignancy. These include simple enucleation, polar segmental nephrectomy, wedge resection, transverse resection, and extracorporeal partial nephrectomy with renal autotransplantation. All of these techniques require adherence to basic principles of early vascular control, avoidance of ischemic renal damage, complete tumor excision with free margins, precise closure of the collecting system, careful hemostasis, and closure or coverage of the renal defect with adjacent fat, fascia, peritoneum, or oxycel. Whichever technique is employed, the tumor is removed with a surrounding margin of grossly normal renal parenchyma. Intraoperative ultrasound is very helpful in achieving accurate tumor localization, particularly for intrarenal lesions that are not visible or palpable from the external surface of the kidney *(9)*. The argon beam coagulator is a useful adjunct for achieving hemostasis on the transected renal surface. If possible, the renal defect crated by the excision is closed as an additional hemostatic measure. A retroperitoneal drain is always left in place for at least 7 d. An intraoperative ureteral stent is placed only when major reconstruction of the intrarenal collecting system has been performed.

In patients with renal cell carcinoma, partial nephrectomy is contraindicated in the presence of lymph node metastasis, because the prognosis for these patients is poor. Enlarged or suspicious-looking lymph nodes should be biopsied before initiating the renal resection. When partial nephrectomy is performed, after excision of all gross tumor, absence of malignancy in the remaining portion of the kidney should be verified intraoperatively by frozen-section examinations of biopsy specimens obtained at random from the renal margin of excision. It is unusual for such biopsies to demonstrate residual tumor but, if so, additional renal tissue must be excised.

3.2. Complications

A recent study detailed the incidence and clinical outcome of technical or renal-related complications occurring after 259 partial nephrectomies for renal tumors at The Cleveland Clinic *(10)*. In the overall series, local or renal-related complications occurred after 78 operations (30.1%). The incidence of complications was significantly less for operations performed after 1988 and significantly less for incidentally detected vs suspected tumors. The most common complications were urinary fistula formation and acute renal failure. A urinary fistula occurred after 45 of 259 operations (17%). Significant predisposing factors for a urinary fistula included central tumor location, tumor size > 4 cm, the need for major reconstruction of the collecting system, and ex vivo surgery. Only one urinary fistula required open operative repair, whereas the remainder resolved either spontaneously (n = 30) or with endoscopic management (n = 14).

Acute renal failure occurred after 30 of 115 operations (26%) performed on a solitary kidney. Significant predisposing factors for acute renal failure were tumor size > 7 cm, > 50% parenchymal excision, > 60 min ischemia time, and ex vivo surgery. Acute renal failure resolved completely in 25 patients, of whom 9 (8%) required temporary dialysis; 5 patients (4%) required permanent dialysis.

Overall, only eight complications (3.1%) required repeat open surgery for treatment, whereas all other complications resolved with noninterventive or endourologic management. Surgical complications contributed to an adverse clinical outcome in only seven patients (2.9%). These data indicate that partial nephrectomy can be performed safely with preservation of renal function in most patients with renal tumors.

3.3. Nephron-Sparing Surgery with a Normal Opposite Kidney

Although radical nephrectomy remains the standard treatment for localized RCC in patients with anatomically and functionally normal opposite kidney, a growing number of authors are reporting excellent results with nephron-sparing surgery in this setting. A recent article detailed the outcome of NSS in 315 reported patients with unilateral localized RCC and a normal opposite kidney *(11)*. The mean cancer-specific survival rate was 95% at approximately 3 yr of follow-up, and there were only two cases of postoperative tumor recurrence. Significantly, the mean tumor size in most of these reports was <3.5 cm. Clearly, patient selection on the basis of small tumor size was a significant factor accounting for the favorable outcome after NSS in these studies.

In a recent study from The Cleveland Clinic, we reviewed the outcome of NSS in 216 patients with sporadic RCC *(8)*. Our findings confirmed that extended cancer-free survival was significantly improved in patients with small (<4 cm) tumors compared to larger ones. Other factors associated with significantly improved survival were unilateral renal involvement low pathological tumor stage, and the presence of a single tumor. There were no postoperative tumor recurrences and the cancer-specific 5-yr survival rate was 100% in patients with small (<4 cm), unilateral stage T_{1-2} RCC.

The aforementioned data suggested that NSS may be an acceptable therapeutic approach in patients who have a single, small (<4 cm) RCC and a normal contralateral kidney. To test this hypothesis, we conducted a subsequent study wherein the outcome following radical nephrectomy vs NSS was evaluated in 88 patients with a single, small (< 4 cm), localized, unilateral, sporadic RCC *(12)*. The radical (n = 42) and nephron-sparing (n = 46) surgical groups were well matched for patient age, sex, renal function, diabetes, hypertension, tumor size, tumor location, and tumor stage. All patients in both groups had low pathological stage RCC. A single patient in each group developed recurrent RCC postoperatively. The cancer-specific 5-yr survival rate for patients in the radical and nephron-sparing surgical groups was 97% and 100%, respectively. More recently, Lerner and associates from the Mayo Clinic reported the results of a similar study comprising patients with solitary small (< 4 cm) low-stage RCC; the 5-yr cancer-specific survival rate following radical nephrectomy vs NSS was 96% vs 92%, respectively *(13)*. The data from these two studies affirm that radical nephrectomy and nephron-sparing surgery provide equally effective curative treatment for patients with a single small, unilateral localized RCC. These patients may now be considered suitable candidates for nephron-sparing surgery even if the opposite kidney is completely normal.

A related issue is whether the location of the tumor in the involved kidney is a significant factor affecting treatment outcome in patients with a single, small, unilateral, localized, sporadic RCC. To address this issue, we conducted another study wherein tumor characteristics and cancer-free survival were compared in patients with centrally vs peripherally located RCCs fulfilling the above criteria *(14)*. The study comprised 145 patients treated with either radical nephrectomy or NSS, and the mean postoperative follow-up was 4.3 yr. Pathological tumor stage was T1-2 in 94% and 82% of central vs peripheral RCCs, respectively. Postoperatively, when comparing patients with central vs peripheral RCCs, there was no difference in 5-yr cancer-specific survival (100% vs 97%), tumor recurrence (5.7% vs 4.5%) or renal function (mean serum creatinine 1.43 mg/dL in both groups). These parameters were also equivalent in patients treated with NSS vs radical nephrectomy both overall and within the central vs peripheral RCC subgroups.

The results of the above study indicate that there are no significant biological differences between centrally vs peripherally located small, solitary, unilateral RCCs. Treatment with NSS or radical nephrectomy is equally effective regardless of tumor location in these patients. From a surgical standpoint, small intrarenal centrally located tumors can be safely and completely removed with temporary renal arterial occlusion and surface hypothermia. Intraoperative ultrasonography is an important adjunct in such cases by providing accurate intrarenal localization of the tumor and thereby enabling its precise excision with a surrounding margin of normal tissue *(9)*. These cases are admittedly more technically demanding than nephron-sparing removal of peripheral tumors and, if this is a concern, radical nephrectomy remains an acceptable treatment option when the contralateral kidney is normal.

3.4. NSS for Advanced RCC

Relatively few studies have examined the role of partial nephrectomy in patients with locally extensive or metastatic RCC. Angermeier et al. reviewed nine patients who underwent partial nephrectomy for RCC with venous involvement in a solitary functioning kidney *(15)*. In all cases, resection of the tumor and preservation of renal function were achieved. A total of five patients were alive without disease at a mean follow-up of 33.2 mo, whereas four died of metastatic RCC, including two who also had local tumor recurrence in the renal remnant. These data suggest an increased incidence of postoperative tumor recurrence when partial nephrectomy is done for localized RCC with venous involvement.

More recently, we reviewed the clinical outcome in 13 patients with metastatic RCC who underwent partial nephrectomy and resection of all metastatic lesions *(16)*. Eight patients had previously undergone a contralateral nephrectomy for RCC and complete resection of one or more metastatic lesions. They all presented for treatment of RCC in their remaining kidney with no other evidence of disease. Five patients (62%) are currently alive and disease-free with mean follow-up intervals of 43 mo from the time of partial nephrectomy and 92 mo from the detection of metastatic disease. Three patients expired at mean follow-up intervals of 57 mo from the time of partial nephrectomy and 72 mo from the detection of metastatic disease. The remaining five patients in this study underwent partial nephrectomy for RCC in a solitary kidney with concomitant complete resection of one or more metastatic lesions. Three of these patients are alive and disease-free (mean 21 mo) and two have expired (mean 20 mo). These data suggest that partial nephrectomy can provide effective treatment for selected patients with RCC and completely resected metastatic disease. The role of adjunction immunotherapy in this setting requires further evaluation.

3.5. NSS in von Hippel-Lindau Disease

RCC in von Hippel-Lindau disease (VHLD) differs from its sporadic counterpart in that the diagnosis is made at a young age, and there usually are multiple bilateral renal tumors. Although these are generally low-stage tumors, they are capable of progression with metastasis and represent a frequent cause of death in patients with VHLD *(17)*. Histopathologically, RCC in these patients is characterized by both solid tumors and renal cysts that contain either frank RCC or a lining of hyperplastic clear cells representing incipient RCC. Therefore, adequate surgical treatment of localized RCC in VHLD requires excision of all solid and cystic renal lesions.

The surgical options in patients with bilateral RCC and VHLD comprise bilateral nephrectomy and renal replacement therapy or partial nephrectomy to avoid end-stage renal failure. Whereas the early results of partial nephrectomy were promising *(18)*, subsequent studies suggest a high incidence of postoperative tumor recurrence in the remaining portion of the kidney *(19)*. It is likely that most of these local recurrences were a manifestation of residual microscopic RCC that was not removed at the time of the original partial nephrectomy.

A recent multicenter study has further delineated the outcome following surgical treatment of localized RCC in 65 patients with VHLD managed at eight medical centers in the United States *(20)*. RCC was present bilaterally and unilaterally in 54 and 11 patients, respectively. Radical nephrectomy and partial nephrectomy were performed in 16 and 49 patients, respectively. The mean postoperative follow-up interval was 68 mo. The 5-yr and 10-yr cancer-specific survival rates for all patients were 95% and 77%, respectively. The corresponding rates for patients treated with partial nephrectomy were 100% and 81%, respectively. In the latter group, 25 patients (51%) developed postoperative local tumor recurrence, however, only two of these patients had concomitant metastatic disease; survival, free of local recurrence, was 71% at 5 yr, but only 15% at 10 yr.

The results of this study indicate that partial nephrectomy can provide effective initial treatment for patients with RCC and VHLD. These patients must be followed closely because most will eventually develop locally recurrent RCC with the concomitant need for repeat renal surgery. When removal of all renal tissue is necessary to achieve control of malignancy, renal transplantation can provide satisfactory replacement therapy for end-stage renal disease *(21)*.

4. FOLLOW-UP AFTER RADICAL NEPHRECTOMY AND NSS

In properly selected patients, both radical nephrectomy and NSS yield excellent long-term patient survival, free of cancer, particularly for low-stage RCC. Yet, there has been no consensus on a standard surveillance protocol following these operations in patients with localized RCC.

We recently completed a detailed analysis of tumor recurrence patterns after partial nephrectomy for sporadic localized RCC in 327 patients at the Cleveland Clinic *(22)*. The purpose of this study was to develop appropriate guidelines for long-term surveillance after partial nephrectomy for RCC. Recurrent RCC after partial nephrectomy occurred in 38 patients (11.6%) including 13 patients (4.0%) who developed local tumor recurrence (LTR) and 25 patients (7.6%) who developed metastatic disease (MD). The incidence of postoperative LTR and MD according to initial pathological tumor stage was as follows: 0% and 4.4% for T_1NOMO RCC; 2.0% and 5.3% for T_2NOMO RCC; 8.2% and 11.5% for T_3aNOMO RCC; and 10.6% and 14.9% for T_3bNOMO RCC. The peak postoperative intervals for developing LTR were 6-24 mo (in T_3 RCC patients) and >48 mo (in T_2 RCC patients).

The above data indicate that surveillance for recurrent malignancy after partial nephrectomy for RCC can be tailored according to the initial pathological tumor stage. The recommended surveillance scheme is depicted in Table 2. All patients should be evaluated with a medial history, physical examination, and selected blood studies on a yearly or twice yearly basis. The latter should include serum calcium, alkaline phosphatase, liver function tests, blood urea nitrogen, serum creatinine, and electrolytes. A 24-h

Table 2
Postoperative Surveillance After Partial Nephrectomy for Localized RCC

Pathological Tumor Stage	*History, Exam Blood Tests*	*Chest X-Ray*	*Abdominal CT Scan*
T_1NOMO	Yearly	—	—
T_2NOMO	Yearly	Yearly	Every 2 yr
T_3NOMO	Every 6 mo for 3 yr, then yearly	Every 6 mo for 3 yr, then yearly	Every 6 mo for 3 yr, then every 2 yr

Table 3
Postoperative Surveillance After Radical Nephrectomy for Localized RCC

Pathological Tumor Stage	*History, Exam Blood Tests*	*Chest X-Ray*	*Abdominal CT Scan*
T_1NOMO	Yearly	—	—
T_2NOMO	Yearly	Yearly	Every 2 yr
T_3abcNOMO	Every 6 mo for 3 yr, then yearly	Every 6 mo for 3 yr, then yearly	At 1 yr, then every 2 yr

urinary protein measurement should also be obtained yearly in patients with a solitary remnant kidney to screen for hyperfiltration nephropathy *(23)*. Patients who have proteinuria may be treated with a low-protein diet and a converting enzyme inhibitor agent which appear to be beneficial in preventing glomerulopathy caused by reduced renal mass.

The need for postoperative radiographic surveillance studies after partial nephrectomy varies according to the initial pathological tumor stage. Patients who undergo partial nephrectomy for T_1NOMO RCC do not require radiographic imaging postoperatively in view of the very low risk of recurrent malignancy. A yearly chest X-ray is recommended after partial nephrectomy for T_2NOMO RCC because the lung is the most common site of postoperative metastasis. Abdominal or retroperitoneal tumor recurrence is uncommon in the latter group, particularly early after partial nephrectomy, and these patients require only occasional follow-up abdominal CT scanning; we recommend that this be done every 2 yr. Patients with T_3NOMO RCC have a higher risk of developing LTR and MD, particularly during the first 2 yr after partial nephrectomy, and they may benefit from more frequent follow-up with chest X-ray and abdominal CT scanning initially; we recommend that these be done every 6 mo during the first 3 yr, following which a chest X-ray is done yearly and an abdominal CT scan is done every 2 yr.

Two recent studies on the outcome after radical nephrectomy for localized RCC have also demonstrated that the risk of postoperative recurrent malignancy is stage dependent *(24,25)*. In a study from M.D. Anderson Cancer Center, metastatic RCC after radical nephrectomy occurred in 68 of 286 patients (23.8%) *(24)*. The incidence of MD according to initial pathological tumor stage was as follows: 7.1% for T_1NOMO RCC; 26.6% for T_2NOMO RCC; and 39.4% for T_3NOMO RCC. The chance of developing recurrent malignancy was greatest during the first 3 yr postoperatively.

These data indicate that surveillance for recurrent malignancy after radical nephrectomy for RCC can also be tailored according to the initial pathological tumor stage. The recommended surveillance scheme is depicted in Table 3. All patients should be evalu-

ated with a medical history, physical examination, and selected blood studies on a yearly or twice-yearly basis. For patients with T_1NOMO RCC, routine postoperative radiographic imaging is not necessary because of the low risk of recurrent malignancy. For patients with T_2NOMO RCC, a chest X-ray every year and an abdominal CT scan every 2 yr are recommended. Patients with T_3NOMO RCC have a higher risk of developing recurrent malignancy particularly during the first 3 yr after radical nephrectomy and may benefit from more frequent laboratory and radiographic follow-up as suggested in Table 2.

In patients treated with either partial or radical nephrectomy for RCC, postoperative bone scans, bone plain films, and head CT scans are necessary only in the presence of related symptomatology. The surveillance schemes outlined in this article are cost effective and enable early detection of most cases of recurrent RCC following surgical treatment of localized disease.

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11 Management of Patients with Renal Cell Carcinoma and Vena Caval Thrombi

Chad W. M. Ritenour and Fray F. Marshall

CONTENTS

1. INTRODUCTION

Renal cell carcinoma (RCC) is well known to invade contiguous vascular structures including the renal vein and inferior vena cava. Such invasion poses challenging therapeutic dilemmas, as there is no adequate medical regimen and surgery often is more difficult and fraught with significant risks. Nonetheless, a successful surgical outcome is possible, and tumors with vascular extension can be cured.

Studies of RCC have shown vena caval extension to occur in approximately 4-10% of cases with a strong male predominance *(1–5)*. This encompasses all levels of tumor thrombus from the renal vein ostia to the right atrium of the heart. The difficulty of surgical approach is related to the degree of cephalad extension of the tumor thrombus. Intracaval neoplastic extension may even necessitate cardiopulmonary bypass for a safe and complete operation.

2. PREOPERATIVE PREPARATION

2.1. History and Physical

A thorough preoperative history to evaluate intercurrent health problems is mandatory in all surgical candidates. Anesthesia, and sometimes medical, consultations are needed to optimize conditions and prepare patients for often long procedures with intense hemodynamic shifts. Particular attention should be applied to cardiovascular status, especially when cardiopulmonary bypass is considered.

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Aside from the classic triad (flank pain, palpable mass, and hematuria) associated with RCC, patients with inferior vena cava (IVC) invasion may also have unique presentations. Although these are rare, symptoms can include massive lower extremity edema from IVC occlusion, ascites from Budd–Chiari syndrome, severe congestive heart failure, intestinal malabsorption, varicocele, or engorgement of the abdominal wall veins. Embolization of a portion of the tumor thrombus may also produce signs consistent with pulmonary embolus.

2.2. Radiographic Tests

A full radiographic evaluation of patients with RCC and vascular extension is required prior to any operative intervention. The purpose of this is twofold, to discover any metastatic disease and to attempt to establish the true superior level of the intracaval tumor. This evaluation can be accomplished by a variety of studies including venacavography, ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI).

Venacavography involves the direct injection of contrast dye into the vena cava. Intravascular tumor can be visualized as filling defects in the contrast-filled cava (Fig. 1). However, cavography sometimes requires both antegrade (femoral) and retrograde (basilic) injections to fully delineate tumor extent *(3)* (Fig. 2). Cavography also has the obvious disadvantage of exposing remaining normal renal parenchyma to a potentially nephrotoxic contrast load. Because of its invasive nature, there is also a risk of dislodging intravascular tumor.

Transabdominal ultrasound has been used to assess the IVC for tumor extension. This examination is highly operator-dependent and is also affected by patient body habitus. Recently, *intraoperative ultrasound* has been utilized to help clarify preoperative findings at the time of surgery *(6–7)* (Fig. 3). Newer ultrasound studies including *color flow Doppler* and *transesophageal echocardiography (TEE)* have also proved valuable for diagnosis *(8–9)*. Particularly, color flow Doppler has been used for cases of equivocal CT scans and has shown excellent accuracy for caval tumor *(10)*.

CT scans are now essentially obtained in all cases of RCC. In fact, these studies are most often used to initially diagnose or confirm the finding of tumor and possible metastatic disease (Fig. 4). As technology has progressed, the resolution and speed of these scans has markedly improved *(11)*. Regardless, there has been criticism of CT for its ability to identify the true cephalad extension of tumor thrombus, especially in the intrahepatic vena cava.

MRI has been extremely useful in assessing vascular invasion by renal cell carcinoma *(3)*. Its advantages include the following: noninvasiveness, no requirement for nephrotoxic contrast administration, and the ability to produce images in various planes (Figs. 5 and 6). Specifically, it is noted for its accuracy in assessing superior extent of tumor thrombus *(12)*. It also delineates the tumor within the cava even when there is total caval occlusion. In general, MRI remains the preferred study for caval assessment.

2.3. Metastatic Evaluation

About one-third of patients with IVC tumor extension have at least one metastatic lesion *(13)*. Multiple studies have shown that survival rates are poor with radical surgery in the setting of metastatic disease *(14–15)*. Therefore, a complete workup for metastatic disease is important prior to surgical intervention. Radiologic tests, including bone scans,

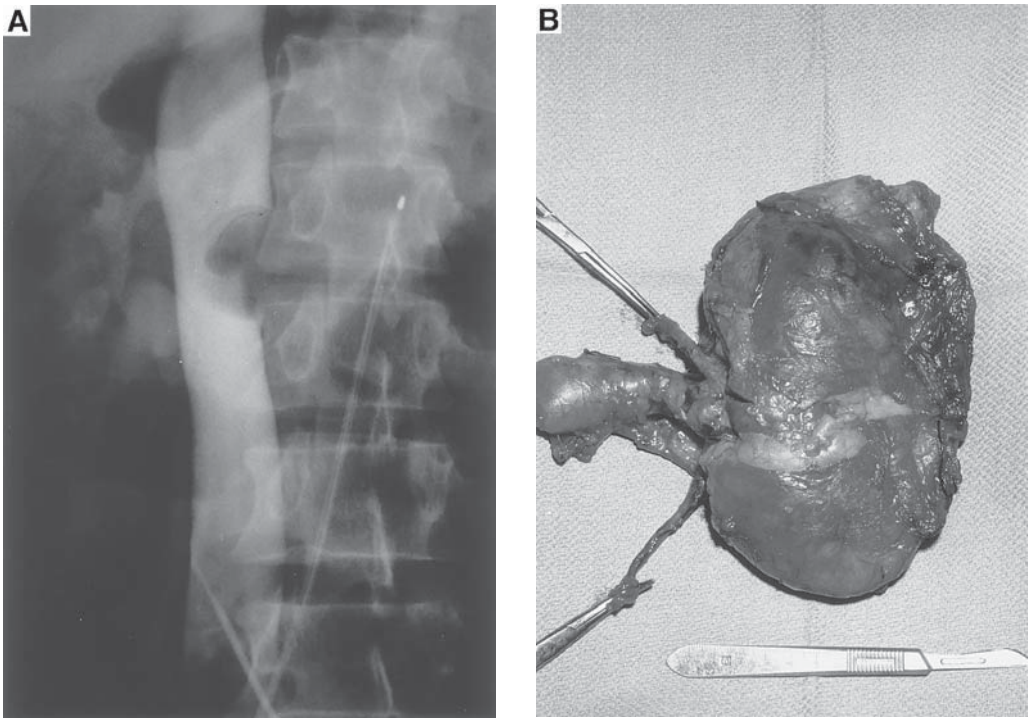


Fig. 1. (**A**) Cavography with filling defect secondary to tumor extension from left renal mass. (**B**) Pathologic specimen with distended left renal vein from tumor thrombus.

again play a valuable role in this setting. Routine serum chemistries and liver function tests may also provide clues to possible disease outside the kidney.

As medical therapy, including immunotherapy, is developed and improved for renal cell carcinoma, there may be a role for surgery in certain cases with metastases *(16–17)*. Several studies report improvement in immunologic response when the primary tumor is removed. Surgical removal of metastatic lesions combined with nephrectomy has also shown to be beneficial *(13–17)*. Nonetheless, thorough knowledge of preoperative lesions will allow much better followup in the postoperative course.

3. OPERATIVE TECHNIQUE

Surgical approach in cases of renal neoplasms extending into the vena cava is dictated by the superior extent of the intracaval or intracardiac tumor. Involvement of intra- and suprahepatic caval levels can create numerous technical problems, thereby lengthening operative time. When sections of vena cava must be resected, reconstruction to allow for adequate venous drainage poses new challenges. Furthermore, extension into the right atrium adds the potential complications of cardiac surgery and cardiopulmonary bypass.

3.1. Approach

Exposure of the neoplastic kidney and associated tumor thrombus can be achieved through several operative approaches. Most cases of RCC with IVC involvement occur on the right side *(3,5)*, but tumors in the left kidney require bilateral dissection. Regardless, full exposure is necessary to ensure complete resection of both tumor and thrombus.

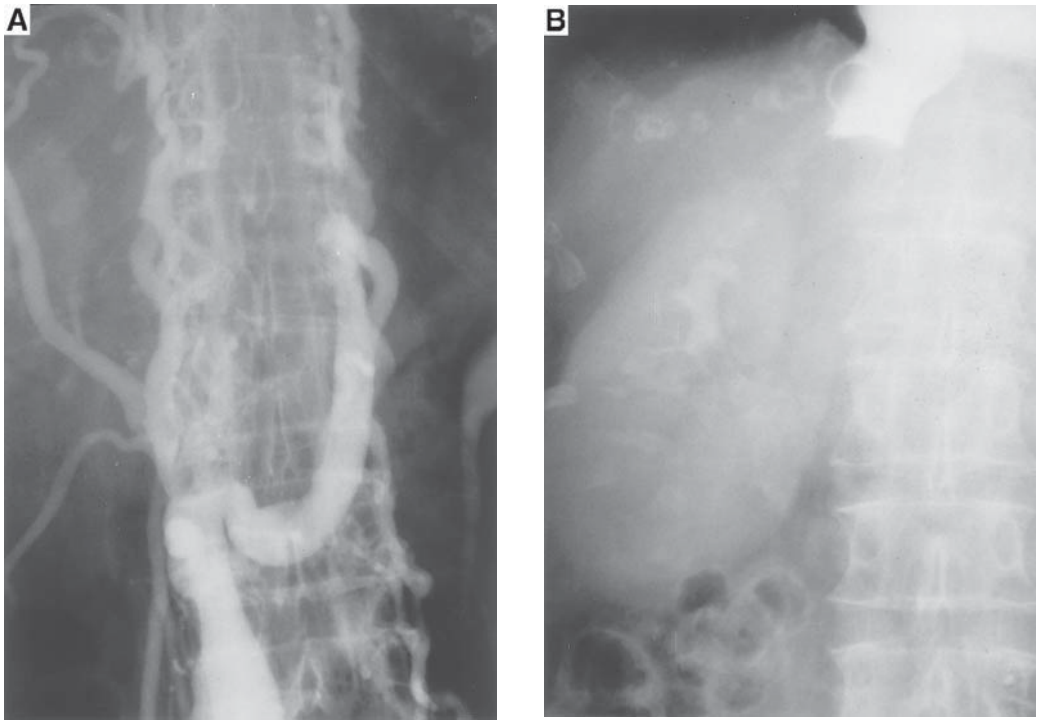


Fig. 2. Antegrade (**A**) and retrograde (**B**) venacavography demonstrating "cutoff" signs marking caval occlusion by tumor.

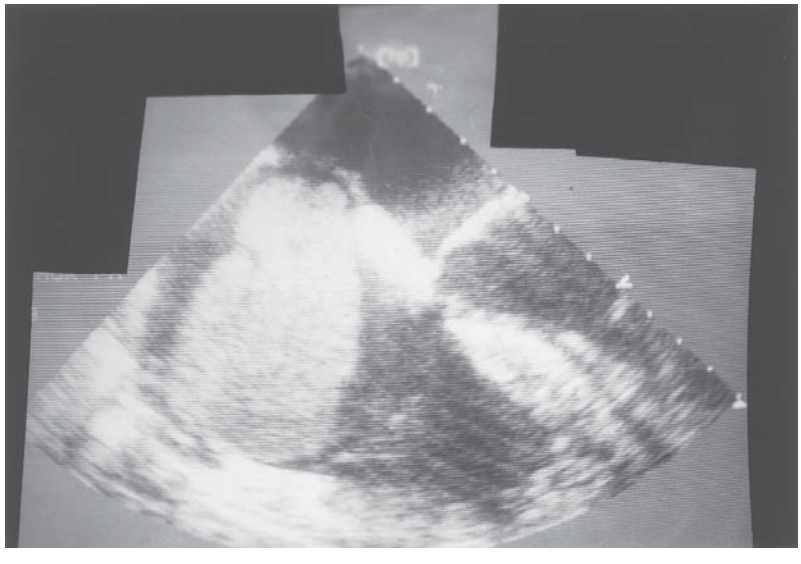


Fig. 3. Intraoperative ultrasound showing intracaval tumor.

The *thoracoabdominal incision* provides excellent exposure for renal tumors, but more limited exposure of the aortic arch for cardiopulmonary bypass. The *midline abdominal incision with extended sternotomy* also allows for excellent visualization. It has the added benefit of more direct access to the heart for cardiopulmonary bypass and hypo-

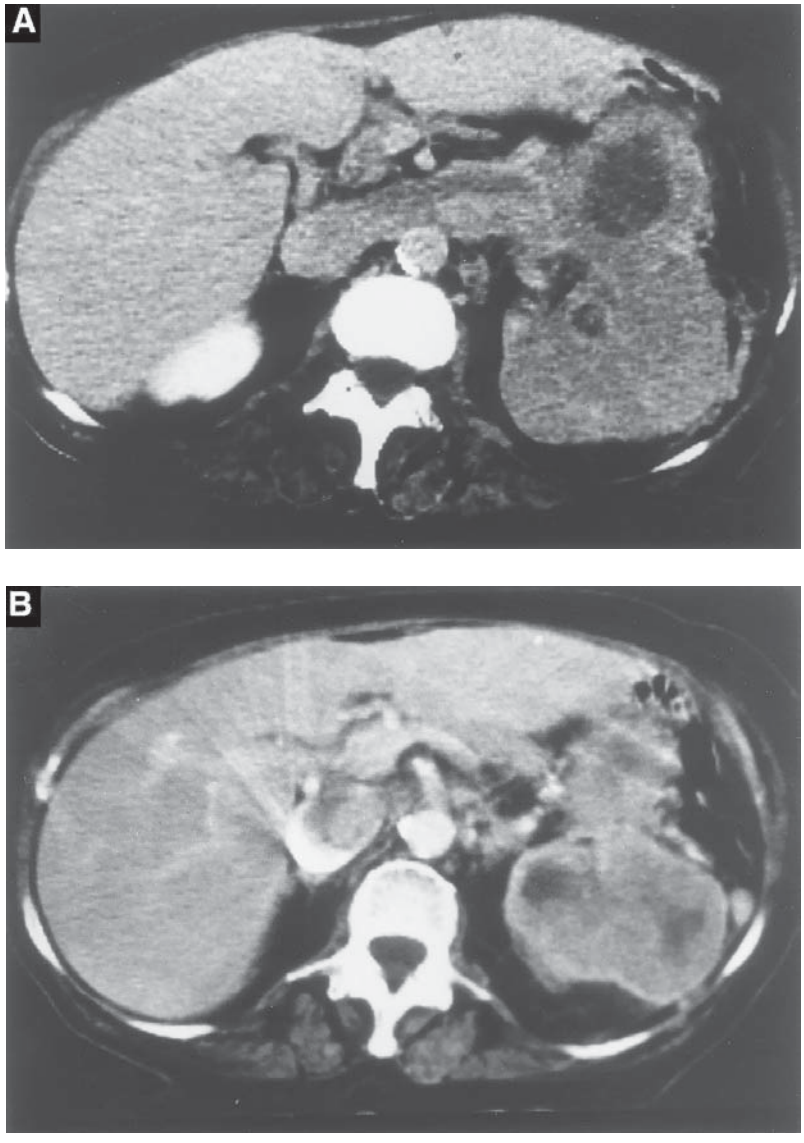


Fig. 4. (**A**) CT scan (axial view) showing large left renal tumor extending through left renal vein into IVC. (**B**) Different image of same patient. Notice tumor in IVC with surrounding contrast.

thermia. However, this approach requires entry into the peritoneal cavity and, therefore, displacement of the bowel. The *chevron incision with extended sternotomy* may be useful for exposure of large tumors in large patients.

3.2. Extent of Resection

The most important goal of surgery for renal cell tumors with IVC extension is complete resection, if possible, as this has tremendous prognostic implications. This includes full removal of caval tumor and resection of any involved caval wall. Following tumor excision, the vena cava can be reconstructed to allow for venous return from other structures including the contralateral kidney.

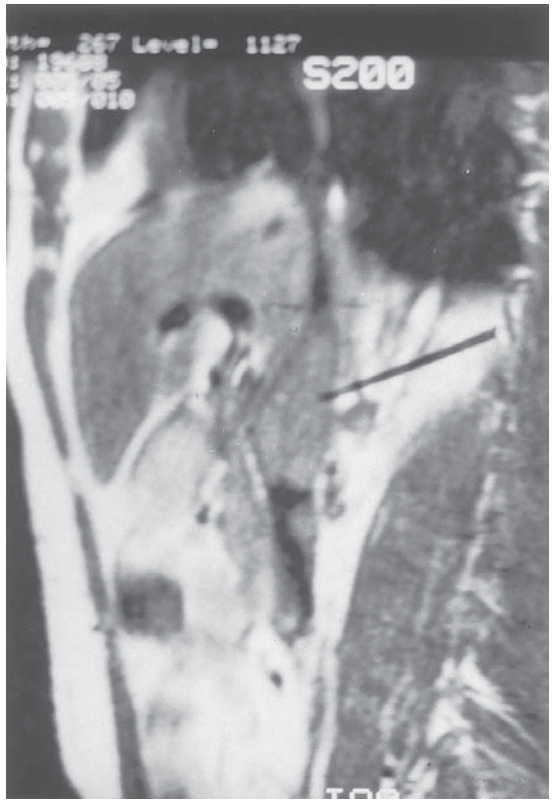


Fig. 5. Coronal MRI view showing sarcomatoid RCC in vena cava.

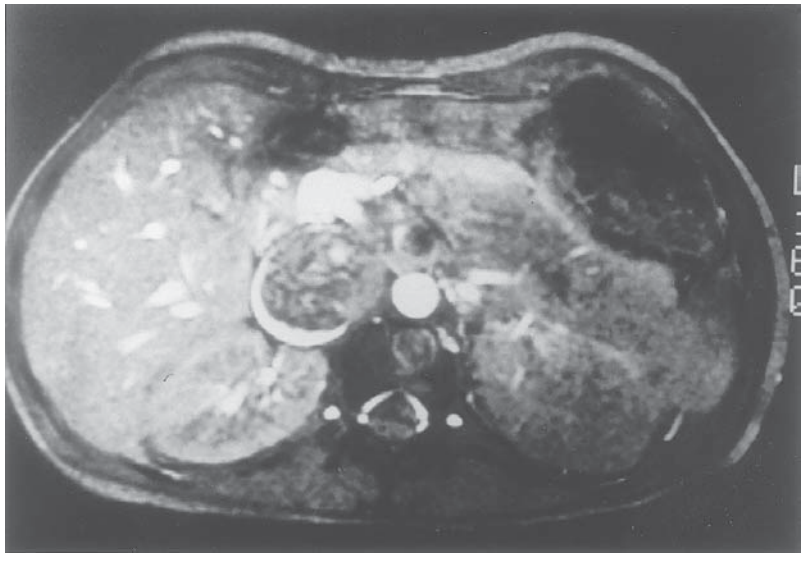


Fig. 6. Axial MRI showing left renal mass with large intracaval tumor near upper pole of right kidney.

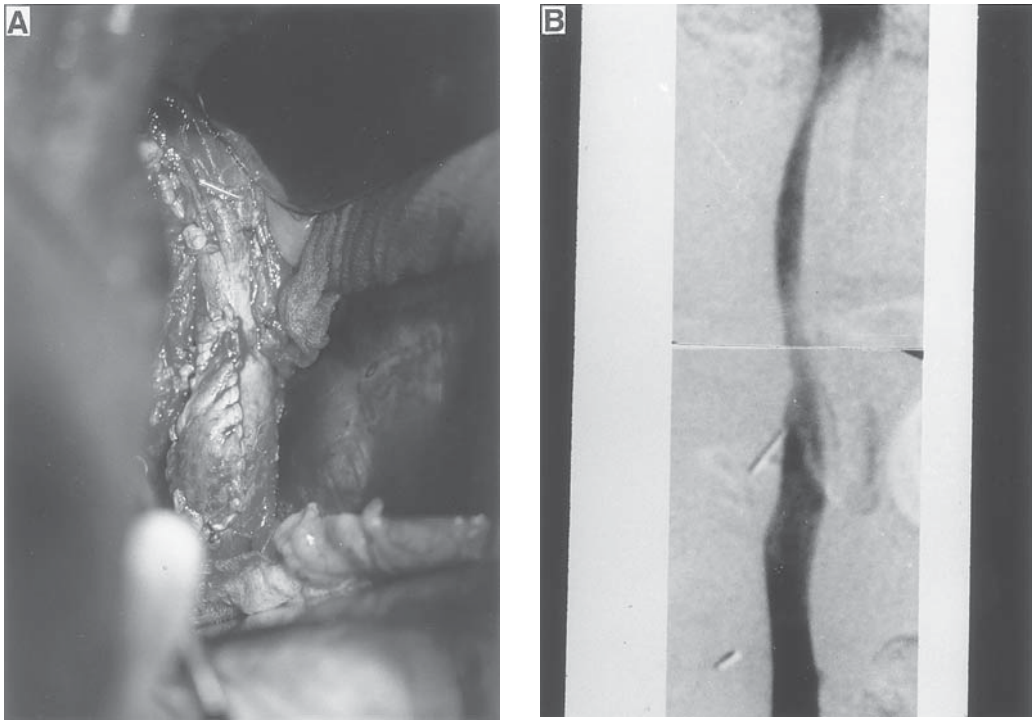


Fig. 7. (**A**) Reconstructed vena cava with pericardial patch following excision of tumor. (**B**) Postoperative cavogram in the same patient.

Tumor extending into the intrahepatic vena cava is often difficult to visualize because of inadequate exposure. Loughlin has described use of the flexible cystoscope placed through a cavotomy incision to assist with visualization of the interior IVC surface at the level of the liver *(19)*. Dental mirrors have also been used for this purpose *(20)*.

Reconstitution of venous drainage after tumor removal depends on the extent of the cavotomy incision. Several studies report that at least 50% of the original IVC lumen diameter must be maintained with caval reconstruction to prevent blood thrombus formation and caval occlusion *(21)*. Occasionally, synthetic graft material is used to construct new portions of the IVC *(22,23)*. The use of pericardium for this purpose has also been described *(21)* (Fig. 7). However, in some situations, segmental cavectomy with ligation of proximal and distal ends has been employed with satisfactory results *(24)*.

For right-sided tumors, the left renal vein can sometimes be ligated if there is good collateral blood flow for venous drainage of the left kidney. Anatomic studies have shown this collateral flow to be mainly dependent on the left ascending lumbar vein, which joins the hemiazygous venous system to drain into the superior vena cava *(2)*. The left ascending lumbar vein receives a branch from the left renal vein, whereas the right ascending lumbar vein bypasses the right renal vein. Because of this finding, the IVC must be reconstructed for left-sided tumors to allow venous return from the right kidney. One Japanese study suggests that the decision regarding reconstruction for right-sided tumors may be based on measuring left renal vein pressures before and after clamping the IVC; if the pressure significantly rises after clamping, then IVC reconstruction should be performed *(25)*.

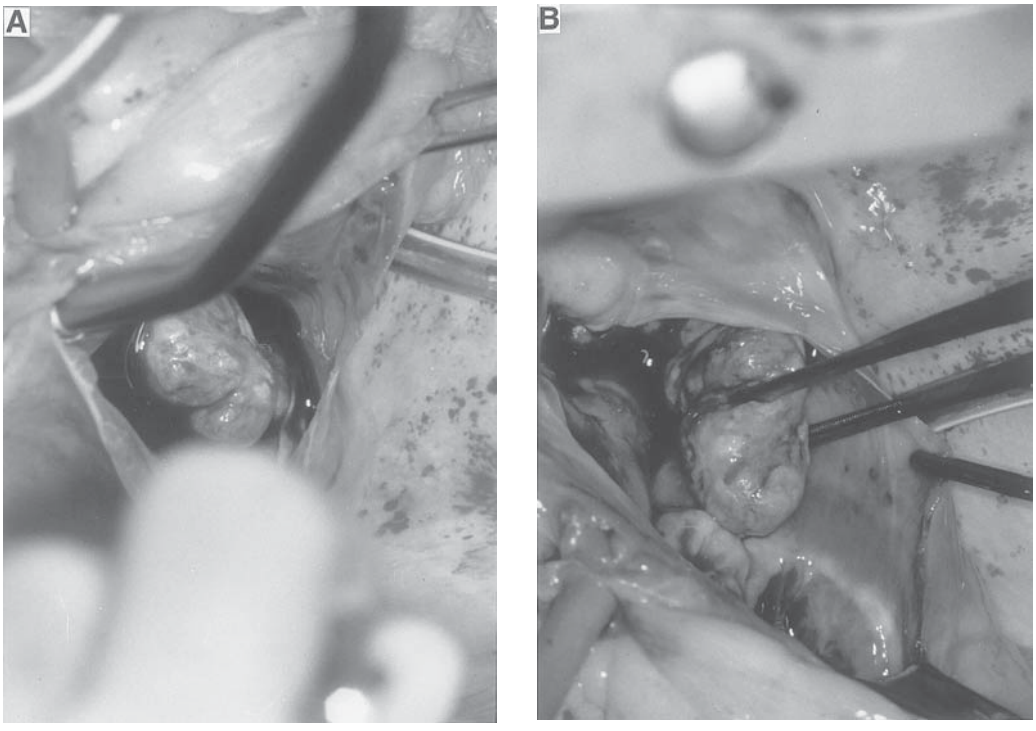


Fig. 8. (**A**) Opened right atrium with visible intracardiac tumor. (**B**) Tumor being dissected from tricuspid valve area in same patient.

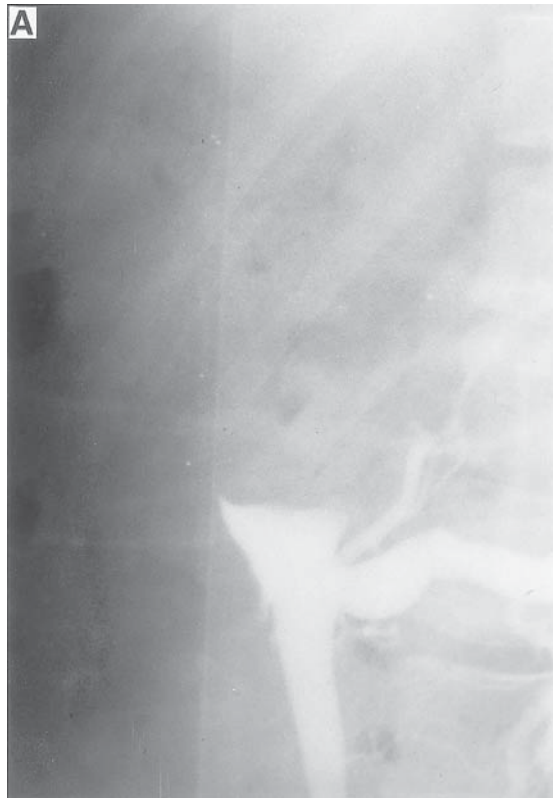
Caval tumor extending into the retrohepatic and suprahepatic IVC requires extensive disssection *(4)*. Significant bleeding can be encountered in posterior mobilization of the liver, particularly the caudate lobe. Some surgeons employ division of the caudate lobe veins to allow for improved exposure of the vena cava and associated thrombus. Even resection of the caudate lobe has been described *(26)*.

3.3. Cardiopulmonary Bypass and Hypothermia

The use of cardiopulmonary bypass and hypothermia has revolutionized surgery for IVC tumor thrombus extending into the right atrium. Bypass allows for an essentially bloodless field, which greatly assists in accessing and viewing pieces of caval and atrial tumor (Fig. 8). Moreover, this helps to decrease risk of tumor embolization during surgical manipulation *(27)*.

To date, use of cardiopulmonary bypass itself has not been shown to decrease survival in cases of renal tumors, but it does allow for full resection of the tumor. Perioperative mortality appears to be related to myocardial dysfunction whereas short-term survival is dependent on lymph node and distant metastases *(28)*. Length of bypass and arrest time also affects survival.

Cannulas are placed in the aorta and right atrium to allow for shunting of blood through the bypass machine (Fig. 9). Systemic heparinization is employed to prevent thrombosis. Once bypass has started, a cardioplegic solution is applied to the heart. To avoid subsequent permanent effects, optimal time for bypass is less than 45 min *(4)*.



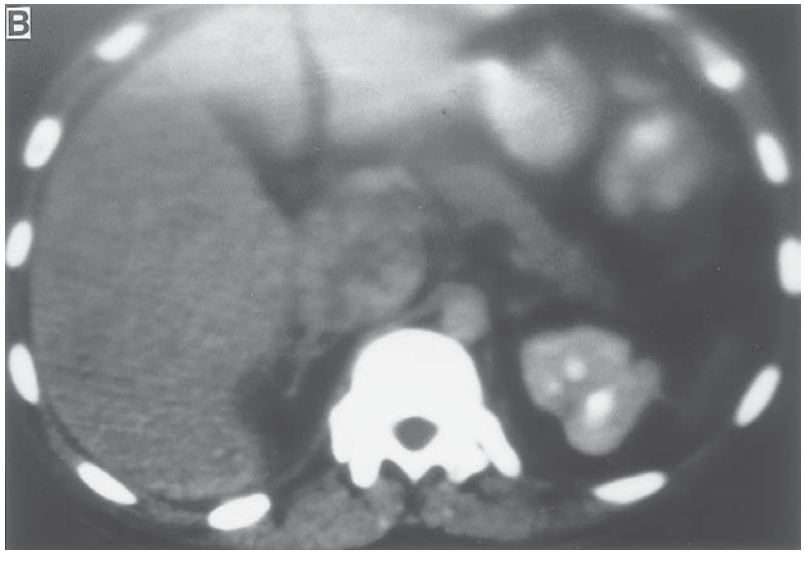
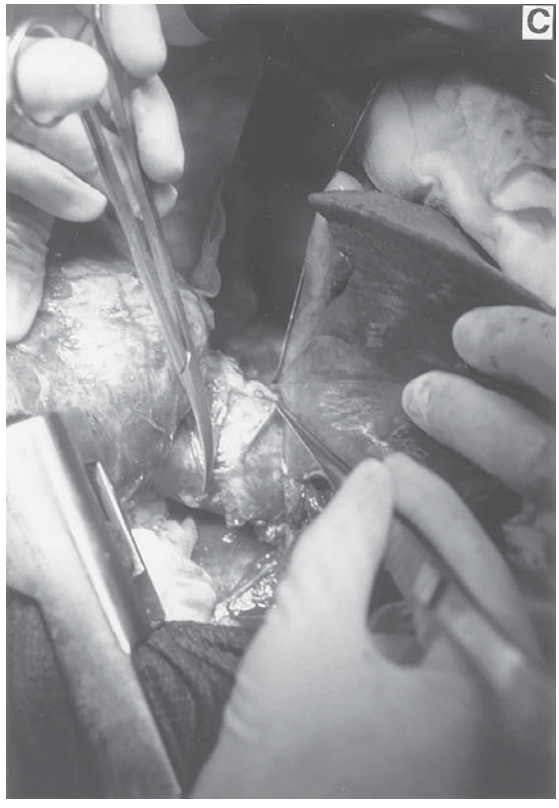


Fig. 9. (**A**) Antegrade venacavogram showing total cava occlusion. (**B**) CT scan in same patient with filling of IVC by tumor.



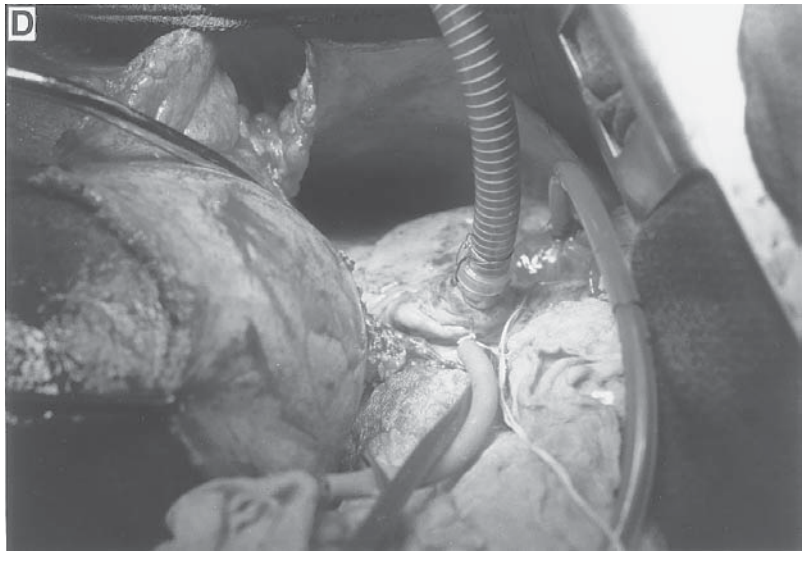


Fig. 9. (Continued). (**C**) Retraction of liver to provide exposure of the inferior vena cava. (**D**) Cannulas in place for cardiopulmonary bypass.

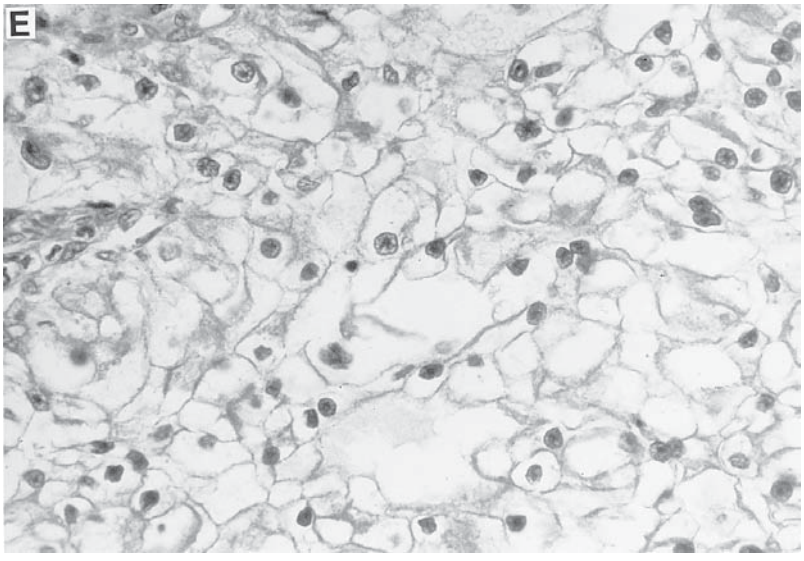


Fig. 9. (Continued). (**E**) Microscopic pathology demonstrating RCC.

Recently, a technique of minimally invasive bypass has been described *(29)*. In this approach, a parasternal incision is made for cardiac exposure. This mirrors trends in coronary artery bypass and valve surgery and decreases morbidity for this part of the operation. As reported, it also avoids repeat sternotomy in previous cardiac surgery patients.

4. POSTOPERATIVE COURSE

4.1. Immediate Complications

Perioperative morbidity and mortality has been well described with surgery involving tumor in the inferior vena cava. Pulmonary embolization of tumor thrombus is a well-recognized complication that sometimes can be avoided by preoperative or intraoperative placement of a vena caval (e.g., Greenfield) filter *(30,31)*. However, a Greenfield filter can itself complicate surgery and pose additional risks (e.g., IVC occlusion or renal vein compromise) *(31)*.

Operative blood loss can be massive, and significant hemorrhage can produce hemodynamic and cardiac effects. Coagulopathy can sometimes occur with prolonged cardiopulmonary bypass. If adequate venous drainage is not ensured for the contralateral kidney, renal failure can ensue.

4.2. Prognosis

A variety of studies have looked at characteristics of RCCs with tumor thrombi to assess prognostic factors both preoperatively and postoperatively from pathologic diagnosis. However, many of these studies contradict others examining the same factors. Several questions have, therefore, not been completely answered, and confusion still exists. There is almost complete agreement that incomplete resection of tumor thrombus portends a much worse prognosis than total removal.

4.2.1. Pathologic Stage of Tumor

4.2.1.1. Venous Extension. Whether or not tumor extension into the vena cava alone affects prognosis has been examined in several studies. In a retrospective analysis of 71 cases, Cherrie et al. showed little prognostic impact from isolated caval extension (median survival = 81 mo) *(32)*. Ljungberg, however, reported a significant survival advantage for patients with tumors confined to the kidney versus those with venous extension *(33)*. Most would agree that survival is more affected by associated tumor factors than venous extension alone.

4.2.1.2. Invasion of the Vein Wall. Microscopic invasion of the vein wall by tumor cells has been reported as the single most relevant prognostic factor for RCC by Van Poppel et al. These investigators estimate a 45% chance of disease progression within one year of nephrectomy in this group of patients *(34)*. Hatcher also reports that a significant improvement in survival is noted for patients for freely mobile as compared to invading tumor (69% vs 26% five-year survival, respectively) *(35)*. Of particular note, resection of the involved caval wall may improve survival *(35)*. Nonetheless, in one series of 26 patients, there was no survival difference between those patients with and without venous wall involvement *(33)*.

4.2.1.3. Lymph Node Invasion. Spread to regional lymph nodes has repeatedly been shown to be a poor prognostic indicator for RCC *(36,37)*. Kuczyk found a significant decrease in life expectancy in this group when compared to a full cohort of patients with tumors invading the vena cava (13 vs 32 mo median survival; $p < 0.001$) *(36)*. Likewise, Nesbitt et al. showed that five-year survival in a group of 37 patients with IVC tumor extension was 0% for the subset with lymph node metastases (versus 33.6% overall) *(38)*.

4.2.1.4. Distant Metastases. Multiple studies have confirmed that the preoperative or intraoperative demonstration of distant metastatic disease leads to a poor prognosis, even with radical nephrectomy *(14,32,36)*. Such patients have much lower long-term survival rates, usually less than 15%, when compared to those without metastases *(5,14,39,40)*.

4.2.2. Level of Tumor Thrombus

Most studies have shown that the cranial extent of tumor thrombus alone has no bearing on prognosis in terms of survival *(33,35,36,41)*. However, several investigators report that higher extending thrombi are associated with significantly decreased survival rates *(40,42)*.

4.2.3. Survival Rates

For cases of caval thrombi from renal cell carcinoma, five-year survival rates have ranged from approximately 30–60% following surgery (Table 1). Again, this is dependent on the factors listed above. The presence of lymph node or distant metastases is associated with much lower long-term survival rates whereas the superior anatomic extent of the thrombus often does not appear to significantly affect them.

The propensity for venous extension of RCC is well established. Management of such cases can be quite difficult, but excellent long-term survival has been achieved with complete surgical removal. Until medical therapy with adequate response rates is developed for this disease, surgery will remain the primary method of cure. Because of this, the preoperative evaluation to select suitable surgical candidates will have the greatest impact on survival rates.

Table 1
Five-Yr Survival Rates in Selected Studies of Renal Cell Carcinoma with Caval Extension

Investigator (Year)	*Number of Patients*		*Isolated venous extension*	*Perinephric fat invasion*	*Metastatic spread, unspecified*	*Lymph node invasion*	*Distant metastases*	*Five-Yr survival*
Pritchett (1986) *(43)*	25							28%
		8	X					33%
Libertino (1987) *(5)*	44							44%
		32[a]	X	X				69%
		5				X		0%
		7					X	0%
Belis (1990) *(37)*	15		X					47%
	16			X				54%
	18					X		12%
Hatcher (1991) *(35)*	44							42%
		27	X					69%[b]
		17			X			18%
Suggs (1991) *(15)*	26							
		21	X					57%
		5			X			0%
Swierzewski (1994) *(14)*	100							54%
		72	X					64%
		28			X			20%
Glazer and Novick (1996) *(41)*	18[c]							57%
Nesbitt (1997) *(38)*	37							34%
			X					45%
					X			25%
Babu (1998) *(44)*	15							55%

[a] "Ideal" (isolated venous involvement) and "favorable" (local extension) candidates.
[b] Freely mobile tumor (Five-yr survival = 26% with pathologic evidence of venous wall invasion).
[c] All patients with intracardiac tumor.

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12 Laparoscopic Surgery for Renal Cell Carcinoma

Inderbir S. Gill

CONTENTS

1. INTRODUCTION

Laparoscopic techniques have been applied to the management of renal cell carcinoma (RCC) since the early 1990s. The seminal contribution in this regard has been Clayman and colleagues' initial description, in 1991, of the technique of laparoscopic nephrectomy *(1)*. Initially, laparoscopic techniques were restricted to simple nephrectomy for benign disease. With increasing experience, however, renal cell cancer has also been approached laparoscopically. Herein, we present the Cleveland Clinic experience with laparoscopic renal surgery for RCC. For the purposes of this chapter, laparoscopic techniques for renal cell cancer will be limited to two primary categories: (1) laparoscopic radical nephrectomy, and (2) laparoscopic renal cryoablation.

2. LAPAROSCOPIC RADICAL NEPHRECTOMY

Laparoscopic radical nephrectomy is being performed only at select centers worldwide *(2–7)*. The majority of these cases are performed by the transperitoneal approach *(3–5)*. At the Cleveland Clinic, we have employed the retroperitoneal approach to laparoscopic radical nephrectomy *(6)*. Although, described by a few authors *(4,7)*, the retroperitoneoscopic approach to radical nephrectomy has not found uniform acceptance because

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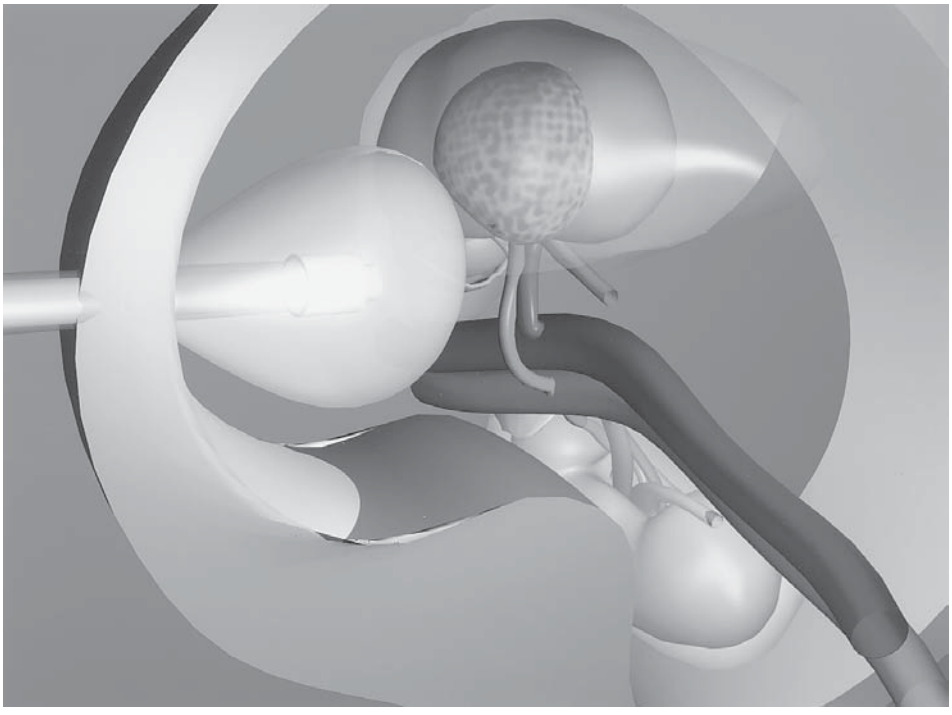

Fig. 1. Balloon dilation of the retroperitoneum to create an adequate working space for laparoscopic manipulations. Note that the balloon is dilated outside of and posterior to Gerota's fascia.

of the smaller area of the retroperitoneal space, the larger size of the radical nephrectomy specimen, and the perceived technical difficulty of en bloc adrenalectomy. However, the retroperitoneal approach has important potential advantages, including early control of the renal vessels and nonviolation of the peritoneal cavity, with resultant minimal paralytic ileus and rapid recovery.

Presented herein is our single-institutional experience with 53 retroperitoneal laparoscopic radical nephrectomies performed in 47 patients. The operative technique is detailed, and our intermediate-term results are presented. Based on these data, we believe that the retroperitoneoscopic approach is an effective technique for performing laparoscopic radical nephrectomy in select patients with renal cancer.

3. TECHNIQUE

Following informed consent, the bowel is prepared with two bottles of magnesium citrate administered the evening before surgery. Under general anesthesia, the patient is secured to the operating table in the standard full flank position. All bony prominences are well padded and extremities carefully placed in the neutral position.

A 1.5-cm skin incision is created at the tip of the twelfth rib *(8)*. Flank muscle fibers are bluntly separated, and the thoracolumbar fascia incised to access the retroperitoneum. Blunt finger dissection creates a space for the balloon dilator *(9)* between the psoas fascia posteriorly and the Gerota's fascia anteriorly. The PDB dilator balloon (Origin MedSystems, Menlo Park, CA) is inserted into the dissected space and inflated with 800 cc of air (Fig. 1). The dilator balloon is removed and a 12-mm Bluntip cannula is secured as the

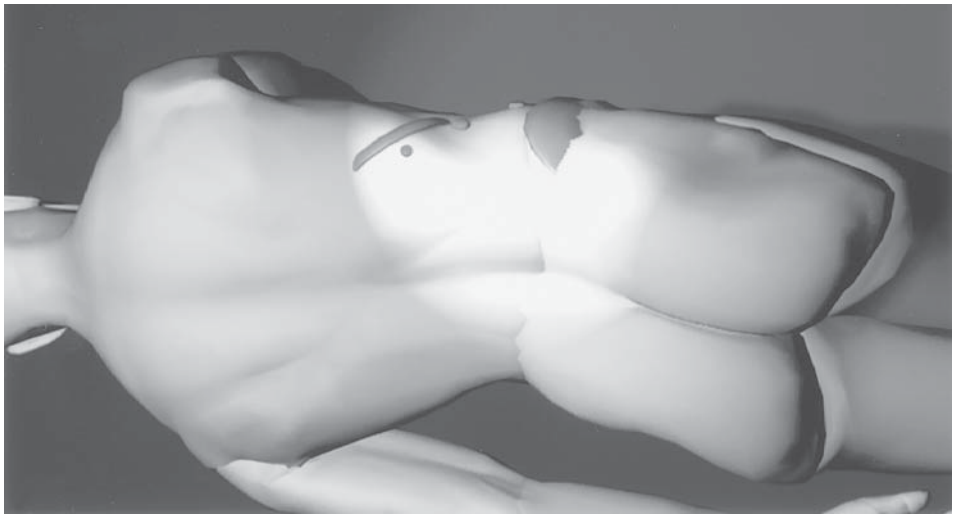

Fig. 2. Port placement for retroperitoneoscopic radical nephrectomy. The posterior port is located at the point where the lateral border of the erector spinae muscles meets the twelfth rib. The middle port is located just below the tip of the twelfth rib. The anterior port is placed near the anterior axillary line, 3 cm cephalad to the iliac crest. Note: The same port placement is employed for laparoscopic retroperitoneal renal cryoablation.

primary port. Pneumoretroperitoneum (15 mm Hg) is created, and two secondary ports are placed under laparoscopic control (Fig. 2).

The renal hilum is accessed through a wide longitudinal incision in Gerota's fascia, parallel and 1–2 cm anterior to the psoas muscle. Renal arterial pulsations are identified, and the artery is individually secured with 11-mm titanium clips (Fig. 3). The renal vein is separately secured with an Endo-GIA vascular stapler. Suprahilar dissection along the medial aspect of the upper pole of the kidney identifies the adrenal vessels, including the main adrenal vein, which are precisely controlled. The specimen, including en bloc adrenal gland, is now bluntly mobilized from the undersurface of the diaphragm and the peritoneal envelope. After the ureter and gonadal vein are secured, the specimen is completely freed by mobilizing the lower pole of the kidney. The entire dissection remains external to the intact Gerota's fascia, thereby mirroring the established oncologic principles of open surgery. The specimen is entrapped within an Endocatch bag (Fig. 4) (Origin Med-Systems, CA) and extracted intact through an enlarged port site incision (Fig. 5). Hemostasis is confirmed, and ports are removed in routine manner. Fascial closure is performed for all 10-mm port sites.

4. RESULTS

Forty-seven patients underwent 53 laparoscopic radical nephrectomies (41 unilateral, 6 bilateral) exclusively by the retroperitoneal approach. On preoperative CT scanning, all patients had localized disease without evidence of perirenal, lymphatic, or renal vein extension. Mean tumor size was 4.6 cm (range, 2–12 cm). Mean patient age was 58 yr (range, 29 to 88 yr) with a mean BMI of 29 (range, 17–63), and American Society of Anesthesiologists Class was 3 (range, 2–4).

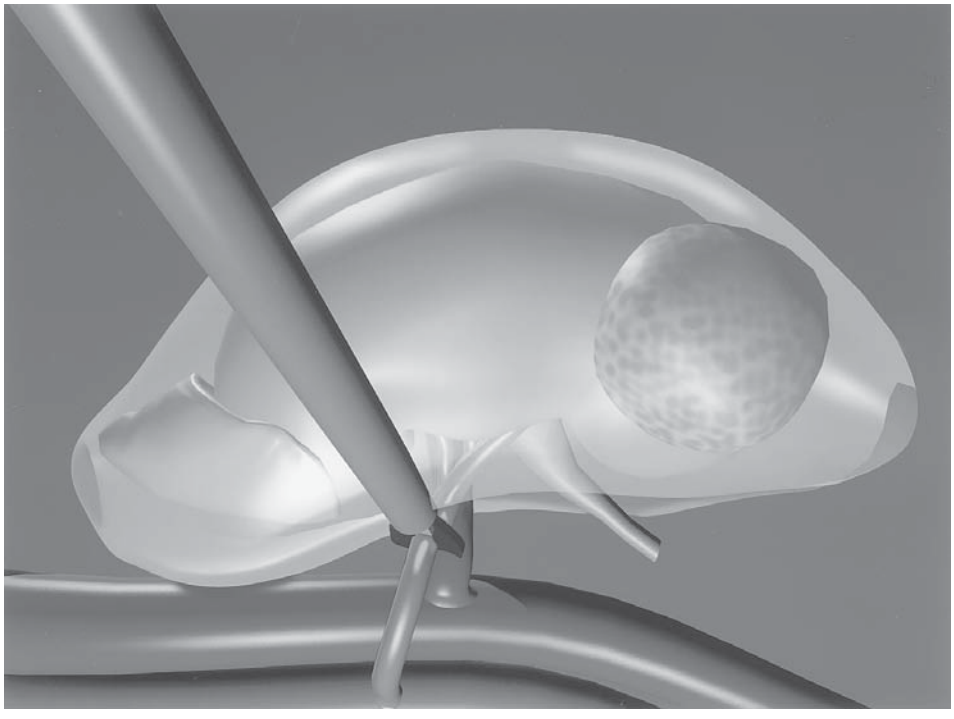

Fig. 3. Renal artery secured with an 11-mm clip applier.

Average surgical time was 2.9 h (range, 1.2 to 4.5 h). Estimated blood loss was 128 mL (range, 20–2000 mL). Mean incision length for extraction of the intact specimen was 6.3 cm (range, 1.5–11 cm). Concomitant en bloc adrenalectomy was performed in 72% of the specimens. In all 16 patients where an adrenal-sparing radical nephrectomy was performed, the preserved adrenal gland was confirmed to be normal in shape, size, and location on preoperative CT scan.

Average hospital stay was 1.6 d (range, <23 h to 6 d). Of the 47 patients, 32 (68%) were discharged from the hospital within 23 h of the surgical procedure. Major and minor complications occurred in two (4%) and 8 (17%) patients, respectively. Major complications consisted of open conversion in two patients. A 67-year-old male with end-stage renal disease, on hemodialysis, developed generalized oozing requiring conversion to open surgery. During the open conversion, a splenic capsular injury occurred, and splenectomy was performed. In another patient, hemorrhage was noted following extraction of the cancerous specimen. At open conversion, bleeding from a branch renal artery was identified and controlled. Minor complications (n = 8) included superficial port-site infection (2 cases), spontaneously resolving retroperitoneal hematoma (2 cases), ileus (1 case), atelectasis (1 case), skin rash (1 case), and cutaneous hyperesthesia (1 case). Conversion to transperitoneal laparoscopy in order to complete the surgical dissection was not required in any patient. However, in six patients, an intentional peritoneotomy was created at the end of the procedure, solely to permit entrapment of the large specimen.

Mean specimen weight was 484 g (range, 52–1328 g): 10 specimens weighed <200 g, 19 specimens weighed between 200–600 g, and 18 specimens weighed more than 600 g. On pathologic examination, 44 specimens (83%) were confirmed to be cancer: RCC (42

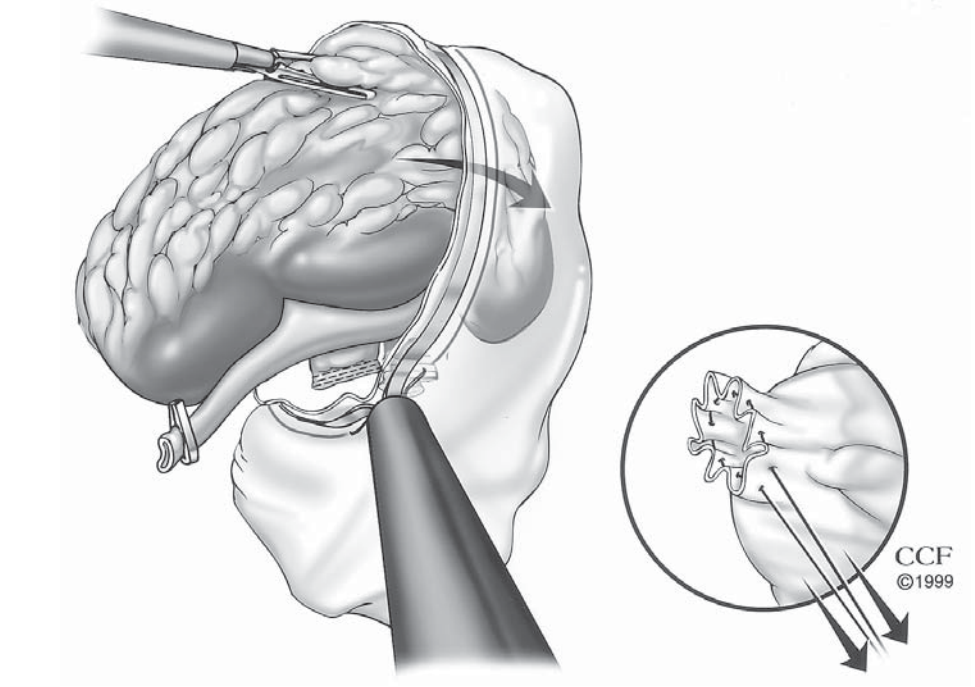

Fig. 4. Specimen entrapment within an Endocatch II device. Inset: Pulling on the built-in drawstring closes the mouth of the sack, thus entrapping the specimen.

cases), transitional cell carcinoma (1 case), leiomyosarcoma (1 case). In 9 instances, there was no evidence of cancer: oncocytoma (3 cases), hemorrhagic cyst (3 cases), angiomyolipoma (1 case), hydronephrosis (1 case), pyelonephritis (1 case).

Over a mean follow-up of 13 mo (range, 1–38 mo), no mortality has occurred to date. Further, no patient has developed a local renal fossa or port-site recurrence. Two patients developed metastatic disease. Both patients were on hemodialysis for end-stage renal disease. A 67-yr-old patient with history of multiorgan cancers (muscle-invasive transitional cell carcinoma of the urinary bladder, prostate adenocarcinoma, bilateral RCC) developed an aortocaval nodal mass 8 mo postoperatively. This patient refused needle biopsy of the mass, and it remains unclear whether this actually represents metastatic lesion, and if so, from which one of his cancers: prostate adenocarcinoma, muscle-invasive bladder transitional cell carcinoma, or bilateral RCC. The second, a 46-yr-old hemodialysis patient, underwent laparoscopic radical nephrectomy for a 4.5-cm right renal mass. A solitary hepatic metastasis was identified 1 yr postoperatively, which was excised open surgically.

We compared the 34 most recent patients undergoing laparoscopic radical nephrectomy (October 1997–March 1999) with a contemporary group of 34 patients undergoing open radical nephrectomy (August 1994–February 1998). Demographic data were comparable between the laparoscopic ($N = 34$) and open ($N = 34$) groups with respect to age, sex, body mass index, and ASA status. No patient in either group had evidence of perirenal infiltration, lymph node enlargement, renal vein and/or caval involvement, or tumor size greater than 12 cm on preoperative CT scan. Mean tumor size was 5 cm in the laparoscopic group and 6.1 cm in the open group ($p = 0.08$). Yet, the weight of the excised

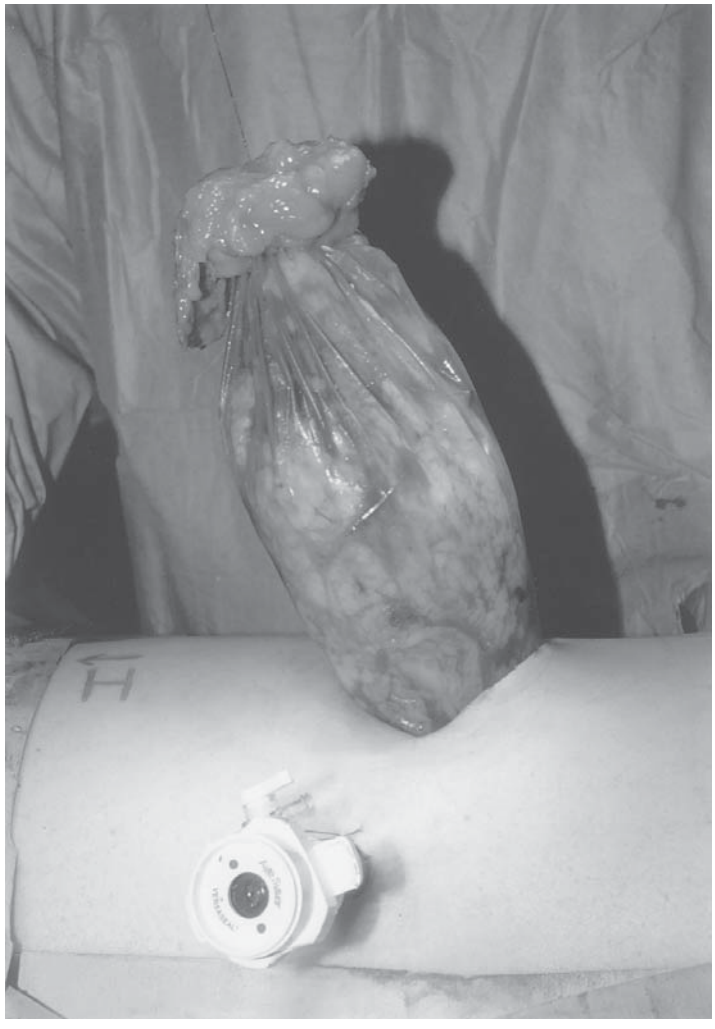

Fig. 5. Laparoscopic radical nephrectomy specimen being extracted intact. Note that the specimen is surrounded by perirenal fat, similar to that obtained by open surgery. This specimen weighed 1.1 kg.

specimens was comparable between the two groups (605 g vs 638 g; $p = 0.93$). The laparoscopic and open groups were comparable regarding surgical time (3.1 h vs 3.1 h; $p = 0.94$). Blood loss was lower in the laparoscopic group (97 cc vs 370 cc; $p < 0.001$). The laparoscopic group was associated with a shorter hospital stay (1.4 d vs 5.8 d; $p < 0.001$), decreased narcotic requirements, and quicker convalescence. Complication rate was 13% in the laparoscopic group and 24% in the open group.

5. DISCUSSION

Laparoscopic radical nephrectomy is currently being performed at a few centers worldwide, commonly by the transperitoneal approach. Barrett et al. of Canada, reported their experience with transperitoneal laparoscopic radical nephrectomy in 72 patients with a mean tumor size of 4.5 cm. Mean operating time was 2.9 h, average specimen weight was 402.5 g, and mean hospital stay was 4.4 d. There was one unexplained intraoperative

death. Six patients (12%) were converted to open surgery. Over a follow-up of 21.4 mo, no port-site recurrences were noted *(5)*. Ono's group from Japan reported their 6-yr experience with laparoscopic radical nephrectomy in 91 patients. Average operative time was 4.9 h, blood loss was 300 cc, and convalescence was completed in an average of 3 wk. Open conversion was necessary in five patients. No local or port-site recurrences were noted over a median follow-up of 22 mo. Metastatic disease occurred in three patients at 3, 19, and 61 mo, respectively *(10)*.

The contemporary comparison of laparoscopic and open radical nephrectomy presented herein attests to the effectiveness and efficacy of the laparoscopic approach. The most recent 34 consecutive patients each undergoing the laparoscopic or open approach were compared. Baseline demographic data were comparable between the two groups, including tumor size (5 cm vs 6.1 cm). Of note, the comparable specimen weights (605 g vs 638 g) and surgical times (3.1 h vs 3.1 h) between the two groups testify to the technical efficiency of the retroperitoneal laparoscopic approach. In addition, laparoscopy was associated with a fourfold decrease in blood loss, a fourfold shorter hospital stay, a ninefold decrease in narcotic analgesic requirements, and a slightly more rapid resumption of normal activities. Dunn et al. from Washington University, compared transperitoneal laparoscopic ($N = 61$) and open ($N = 34$) radical nephrectomy. Laparoscopy was associated with increased operative time (5.5 h vs 2.8 h; $p < 0.001$), lesser blood loss (172 mL vs 456 mL; $p = 0.01$), decreased narcotic dosage (27 mg morphine sulfate vs 82 mg; $p < 0.001$), abbreviated hospital stay (3.6 d vs 5.1 d; $p < 0.001$), and quicker convalescence (8.4 wk vs 30.6 wk; $p = 0.002$). Renal cancer recurred in five (13%) patients in the laparoscopic group and three (10%) patients in the open group *(11)*. Similarly, Ono and colleagues from Japan compared laparoscopic ($N = 60$) and open ($N = 40$) radical nephrectomy. Laparoscopy resulted in a longer operative time (5.2 h vs 3.3 h), lesser blood loss (255 mL vs 512 mL), and shorter convalescence (3 wk vs 8 wk). Five-year disease-free incidence was 95.5% in the laparoscopy group and 95.7% in the open group *(10)*.

A five-center report by Caddedu and colleagues recently documented the oncologic efficacy of laparoscopic radical nephrectomy in 157 patients with a clinically localized, pathologically confirmed RCC *(2)*. Over a mean follow-up of 19.2 mo (range, 1–72), no patient experienced a port-site or renal fossa recurrence, or cancer-related death. Metastatic disease developed in four patients. One patient developed a local recurrence in the ureteral stump. The estimated 5-yr actuarial disease-free rate for all patients ($N = 157$) and patients with T_2 disease ($N = 124$) was 91% and 89%, respectively.

In summary, at the Cleveland Clinic, laparoscopic radical nephrectomy is preferentially performed by the retroperitoneoscopic approach. We believe that retroperitoneoscopy offers unique advantages for this procedure. Paramount is the superb and rapid access to the renal artery and vein, which are sequentially secured early during the procedure, prior to any mobilization of the cancerous kidney. Meticulous extra-fascial mobilization of the entire specimen, including en bloc adrenal gland, duplicating open surgical oncological principles, is routinely feasible. We have employed this technique in renal tumors up to 12 cm in size and 1.3 kg in weight. Over a mean follow-up of 13 mo (range, 1–38 mo), no local or port-site recurrence has been noted, and all patients are alive to date. Two patients (4%) developed evidence of metastatic disease.

On the basis of data presented herein, we have expanded our indications, and currently consider as candidates for laparoscopic radical nephrectomy, the majority of patients with a T_1–T_{3a} N_0M_0 renal cancer without evidence of perirenal, lymphatic, or vascular extension.

6. LAPAROSCOPIC RENAL CRYOABLATION

The diagnostic and management dilemma posed by the radiographic detection of a small (<4 cm), incidental, solid or complex cystic renal mass is an increasingly frequent clinical scenario. The reported slow growth rate, low metastatic risk, and preoperatively often questionable diagnosis combine to make the overall significance of such lesions uncertain *(12)*. Depending upon the individual clinical situation, treatment alternatives include watchful waiting, radical nephrectomy, or increasingly, partial nephrectomy. Although the long-term cancer-cure rates and functional efficacy of partial nephrectomy are well documented *(13)*, the procedure itself is associated with the potential morbidity of open surgery.

With the increasing application of minimally invasive surgery, experimental open, percutaneous, and laparoscopic renal cryoablation has been successfully investigated in the laboratory, as well as clinically *(14–19)*. At the Cleveland Clinic, we perform renal cryoablation by the laparoscopic approach. Our indications, surgical technique, and initial results in 32 patients are presented herein.

7. INDICATIONS

For this initial experience, renal cryoablation was offered only to carefully selected patients who fit the following stringent selection criteria: small (≤4 cm), peripheral, preferably exophytic renal tumors, 1 or 2 in number, that are located at a distance from the collecting system.

8. TECHNIQUE

The patient is placed in the full flank position. The table is flexed and kidney bridge elevated. Retroperitoneoscopic access is obtained with balloon dilation by the similar technique as described in the preceding section on laparoscopic radical nephrectomy. A total of three trocars are placed: a 10–12-mm Bluntport at the tip of the twelfth rib, a 12-mm port in the midaxillary line 3 cm superior to the iliac crest, and a posterior 5-mm port at the lateral border of the psoas muscle just below the twelfth rib (Fig. 2).

Using laparoscopic techniques, the kidney is mobilized within Gerota's fascia, thus exposing the entire renal surface, including the tumor. The perirenal fat overlying the tumor is removed for histopathologic examination. Mannitol (12.5 g) is administered intravenously upon commencing renal mobilization. The renal hilum is not dissected.

An endoscopic, steerable, color-Doppler, ultrasound probe is inserted through the 12-mm midaxillary port by a radiologist who has expertise with performing endoscopic ultrasonography. The probe is placed in direct contact with the renal surface (Fig. 6), and detailed ultrasound examination of the entire kidney is performed to evaluate the following: margins, size, and vascularity of the renal tumor, proximity of the tumor margins to the collecting system, and presence of any satellite tumors in the remainder of the kidney. Needle biopsy of the tumor is performed (Fig. 7) with a 15-gage 15-cm needle with echogenic tip (ASAP™ Biopsy System, Order #500-128, Microvasive, Boston Scientific Corp., Watertown, MA), and the tissue sent for routine histopathologic examination.

Puncture cryoablation is performed with a 4.8-mm cryoprobe with a 2- or 4-cm freeze-zone, depending upon the tumor size. With the ultrasound probe placed in direct contact with the opposite surface of the kidney, the conical tip of the cryoprobe is gently inserted into the center of the tumor under laparoscopic visualization. Under real-time ultrasonic

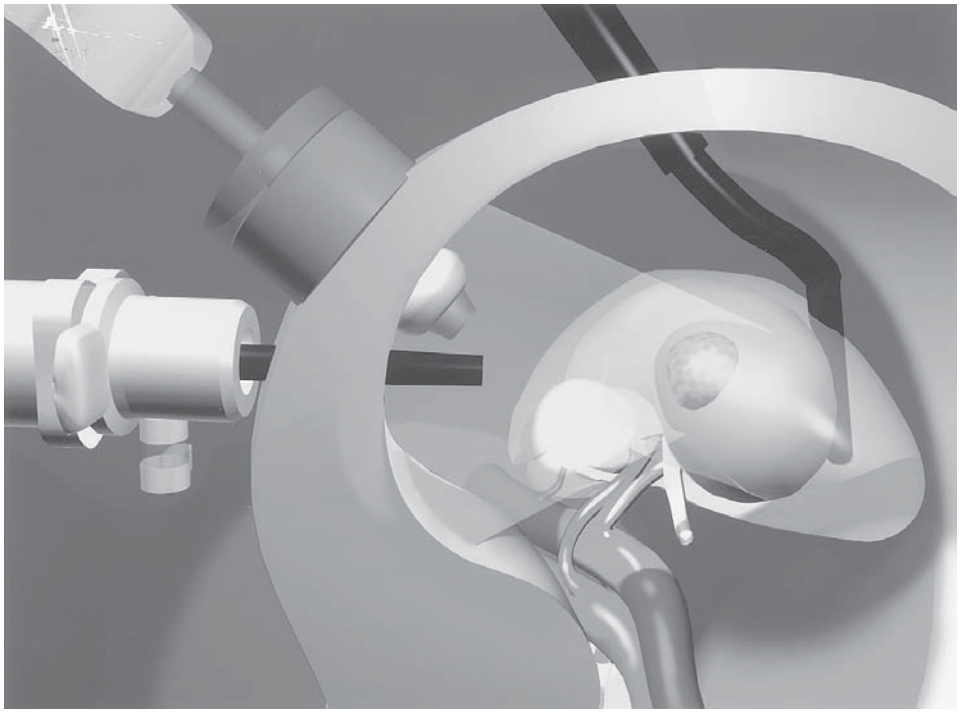

Fig. 6. Real-time, steerable, laparoscopic ultrasonography of the renal tumor. The flexible ultrasound probe is placed in direct contact with the surface of the kidney opposite to the tumor. This allows the inner margin of the evolving ice-ball to be accurately imaged in real-time fashion.

guidance, the tip of the cryoprobe is advanced up to just beyond the deep border of the tumor. Cryoablation is initiated, and a double freeze-thaw cycle is completed under real-time endoscopic ultrasound monitoring (Fig. 8). A rapid freeze is performed (tip temperature –185%C to –195%C) until the leading, hyperechoic, semilunar edge of the ice-ball is noted to circumferentially extend approximately 1 cm beyond the tumor margins on both ultrasonographic and laparoscopic visualization. It is critical to ensure that the entire external ice-ball on the renal surface is completely exposed and under clear laparoscopic visualization at all times. The adjacent peritoneum and ureter must never come into even momentary contact with the ice-ball or the active cryoprobe, to avoid potentially disasterous transmural cryoinjury. After the rapid initial freeze, a slow complete thaw is performed until the ice-ball begins to melt. With the cryoprobe carefully maintained in position, a second rapid freeze is performed. Laparoscopic visualization confirms that the visible external surface area of the ice-ball is similar to that of the first freeze. Because the cryodestruction created by the initial freeze cycle may transiently render the ablated area anechoic, real-time ultrasonography may be unable to visualize the advancing edge of the second ice-ball until it advances beyond the boundary of the initial ice-ball.

On completion of the second thaw, melting of the cryolesion releases the probe, which is removed gently, without torquing. Forceful or premature removal of the cryoprobe may cause cryolesion fracture, and cause post-thaw hemorrhage. Upon removal of the cryoprobe, hemostatic pressure is maintained on the puncture site with a piece of Surgicel for 10 to 15 min. Thereafter, it is important to lower the pneumoretroperitoneum to 5 mm Hg for an additional 5 min to confirm hemostasis. A patch of an absorbable adhesion

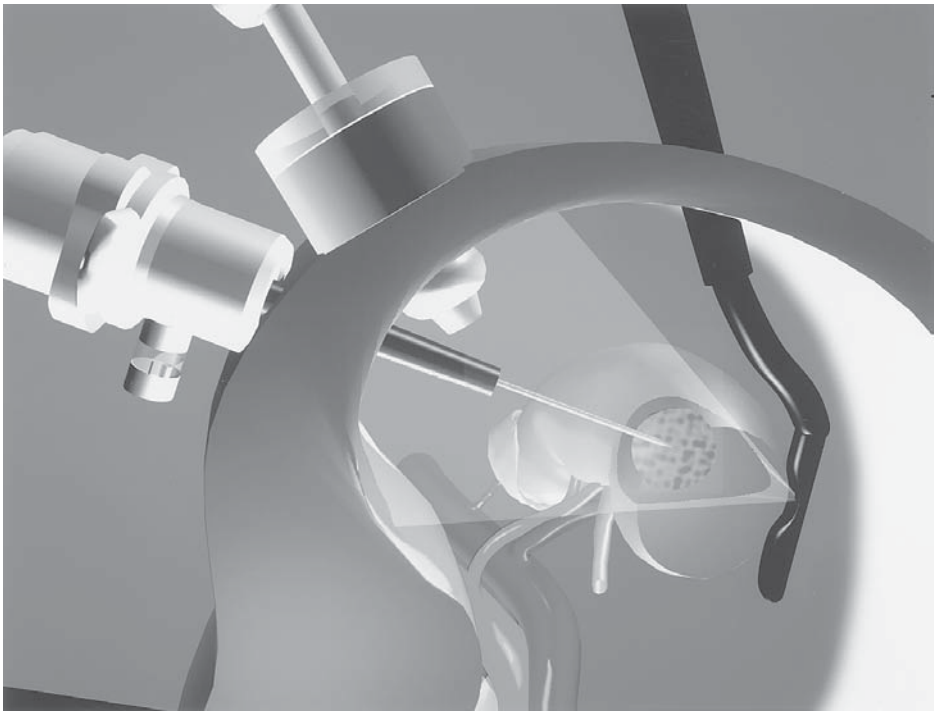

Fig. 7. Needle biopsy of the renal tumor under laparoscopic visualization and ultrasound monitoring.

barrier (Interceed, Johnson & Johnson, Arlington, TX) is placed over the Surgicel to minimize adhesion formation (Fig. 9). We do not drain the operative site. Ports are removed under laparoscopic visualization and closure performed in routine manner.

9. RESULTS

Since September 1997, 27 patients have undergone laparoscopic renal cryoablation at the Cleveland Clinic. All tumors were small (≤4 cm), circumscribed, and removed from the collecting system. Indications for laparoscopic renal cryoablation were as follows: tumor size ≤4 cm (13 patients), solitary kidney *(6)*, contralateral renal cancer *(3)*, renal dysfunction *(2)*, calculus disease *(2)*, and metastasis to kidney *(1)*. Mean patient age was 64 yr (range, 35–93 yr) and mean size of the renal tumor on preoperative CT scan was 2.2 cm.

Laparoscopic renal cryoablation was performed by the retroperitoneal approach in 23 patients and transperitoneally in 4 patients. All procedures were technically successful. Intraoperative precryoablation needle biopsy revealed RCC in 15 patients, atypical cells in two patients, oncocytoma in two patients, angiomyolipoma in one patient, and nondiagnostic in six. Laparoscopic ultrasonography satisfactorily imaged the renal tumor and the evolving cryolesion in all cases. A double freeze-thaw cycle was performed routinely. Cryoablation time averaged 15.1 min. Mean blood loss was 70 cc (range, 10–200 cc). Total surgical time averaged 3 h. A superficial, liver laceration due to trauma from a laparoscopic fan retractor, which resolved spontaneously, was the solitary intraoperative complication.

Oral intake and ambulation were resumed within 24 h by the majority of the patients. Hospital stay averaged 1.7 d. There were two postoperative complications, both managed

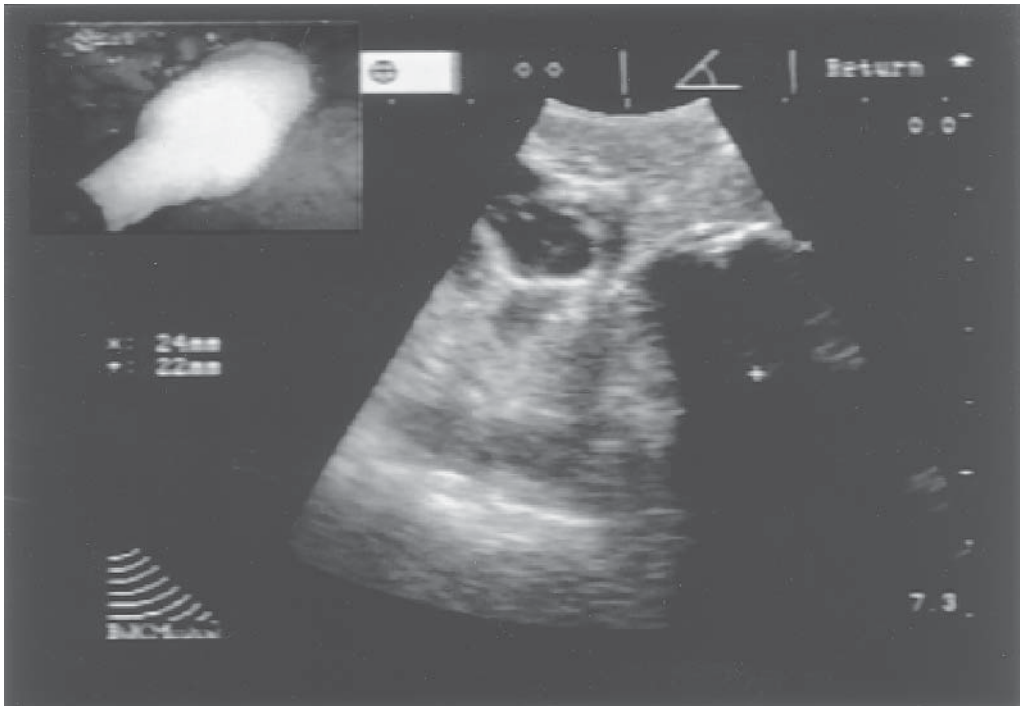

Fig. 8. Ultrasound image of the ice-ball. The semicircular hyperechoic image represents the advancing edge of the ice-ball. The body of the ice-ball is anechoic. Picture-in-picture inset shows a laparoscopic view of the ice-ball.

Fig. 9. Laparoscopic view upon completion of renal cryoablation. Hemostasis is ensured and the cryoablated tumor is covered by Surgicel and Interceed to minimize postoperative adhesions.

conservatively: one patient developed an asymptomatic, perirenal hematoma, whereas another had a late rehospitalization for herpes esophagitis. Convalescence was complete at 2 wk (range, 2 d to 10 mo). Mean pre- and postoperative serum creatinine levels were 1.2 mg% and 1.3 mg%, respectively. Mean preoperative and postoperative serum hematocrit levels were 42 and 37, respectively. Average follow-up is 9 mo (range, 1–17 mo).

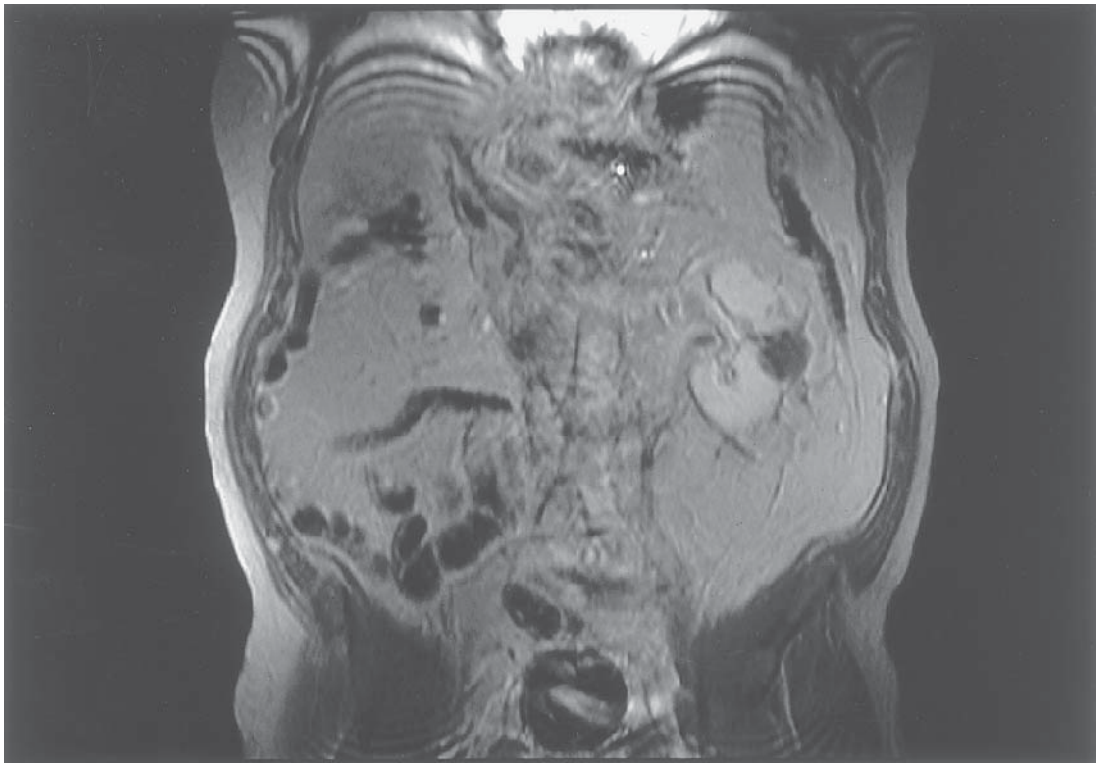

Fig. 10. Postoperative MRI image showing the punched-out circular defect in the left kidney representing the cryoablated tumor.

Follow-up MRI scans of the kidney were performed at 1 d and 1, 2, 3, 6, and 12 mo postoperatively. Nonenhancement of the central portion of the cryolesion was the primary MR hallmark of technically successful cryoablation (Fig. 10). Cryolesions appeared isointense to the adjacent renal parenchyma on T_1-weighted, and heterogeneously enhancing on T_2-weighted images. On gadolinium-enhanced T_1-weighted sequences, the cryolesion was clearly demarcated as a hypointense semicircular area. Occasionally, peripheral rim-enhancement was seen. Cryolesions contracted in size over time. Average cryolesion diameter at 1 day, and 1, 2, 3, and 6 mo was 3.5 cm, 3.2 cm, 2.7 cm, 2.1 cm, and 1.8 cm, respectively. This is the equivalent of a size reduction of 8%, 23%, 40%, and 48% at 1, 2, 3, and 6 mo, respectively.

Of the 7 patients who have completed a 1-yr follow-up, five have undergone MRI scanning at 1 yr. No residual cryolesion can be identified in two patients. In the remaining three patients, mean cryolesion diameter was 1.5 cm, a size reduction of 57%.

To date, 15 patients have undergone a CT-guided needle biopsy of the cryoablated tumor at a follow-up of 3-6 mo postoperatively. Histopathology revealed no evidence of cancer in any of the patients.

10. DISCUSSION

Cryosurgery aims to ablate a comparable, predetermined volume of tissue as would have been excised in the event that a conventional surgical procedure had been performed *(20)*. Critical denominators for successful visceral cryosurgery include rapid freezing, slow thawing, and a repetition of the freeze-thaw cycle *(21)*. Thus, the diseased tissue,

with an adjacent margin of healthy parenchyma is frozen *in situ*. This devitalized tissue is then allowed to slough and autoamputate over time, with granulation healing by secondary intention.

The pathophysiology of cryoablation is thought to involve: (1) immediate cellular injury, and (2) delayed microcirculatory failure. According to Mazur's two-step theory of immediate cellular damage *(22)*, initially, ice forms in the extracellular space, causing the extracellular fluid to become hyperosmotic. In order to equilibrate, water permeates out from the intracellular compartment, thus increasing the osmolality of the intracellular fluid, with resultant intracellular solute concentration, dehydration, and desiccation trauma—the first step of chemical cellular injury. Continued supercooling leads to formation of intracellular ice, the second step of cellular damage. Intracellular ice is a lethal event, irreversibly disrupting cell organelles and the cell membrane.

Microcirculatory failure, a delayed phenomenon, occurs during the gradual thaw phase of the freeze-thaw cycle, culminating in cessation of circulation and cellular anoxia *(23)*. The microcirculation progressively fails along a sequential cascade: vasoconstriction, injury to the endothelial layer causing vessel walls to become porous, interstitial tissue edema, platelet aggregation, microthrombii formation, and ultimately vascular obliteration. Cells that survive the initial assault of freezing are destroyed by this secondary ischemic trauma *(21)*. This damage is intensified by a repetition of the rapid freeze-slow thaw cycle. The ischemic cryoablation area ultimately suffers a circumscribed necrosis.

Renal cryoablation has been performed in the laboratory by open, percutaneous, and laparoscopic techniques *(14–17)*. Complete necrosis of porcine renal parenchyma was demonstrated consistently at temperatures of minus 19.4ºC or lower. However, a higher temperature, ranging between minus 19.4ºC and 0ºC, caused renal necrosis in only 80% of tissue samples *(24)*. Lethal temperature of minus 20ºC could be achieved at a distance of 3.1 mm inside the outer edge of a 3.2-cm ice-ball created by a 3.4-mm diameter cryoprobe *(25)*. Therefore, to achieve uniform cell death, the ice ball must extend well beyond (approx 1 cm) the visible margins of the targeted tumor.

Clinically renal cryoablation has been performed by the open *(19)*, percutaneous *(18)*, and laparoscopic *(26,27)* techniques. The Johns Hopkins group recently presented their experience in nine patients with exophytic renal masses with a mean size of 2 cm. Mean blood loss was 140 cc and hospital stay was 3 d. There were no complications. At a mean radiographic follow-up of 5 mo, no tumor recurrences were evident *(27)*.

Critical questions remain about cryoablation for renal cancer. At this time, the primary criticism is the nonavailability of histologic data about the thoroughness of tumor destruction and status of the surgical margins. Clearly, long-term follow-up, both radiologic and clinical, is necessary to provide information regarding local recurrence and cancer-free survival.

Although laparoscopic renal cryoablation is still developmental, the early results are encouraging. Over a mean follow-up of 9 mo (range, 1 to 17 mo), no patient has had radiologic evidence of systemic recurrence. Cryolesions have decreased in size, as determined by sequential MRI scans. Clearly, needle-biopsy of renal masses is not a completely reliable test, with a reported false-negative rate of 16% *(28)*. Nevertheless, it is encouraging that in all 15 patients biopsied at 3-6 mo following cryoablation, no evidence of RCC has been found in any patient. Meticulous long-term follow-up will be required to definitively determine the efficacy of cryoablation in the treatment of patients with a select, small, incidental renal mass.

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13 Adjuvant Therapy of Renal Cell Carcinoma

Ronald M. Bukowski

CONTENTS

1. BACKGROUND

Renal cell carcinoma (RCC) is a tumor that can be cured by surgical excision, however, this approach fails to prevent recurrence and/or death in a significant proportion of patients. In 1973, Bloom *(1)* noted that 50% of patients undergoing nephrectomy ultimately developed recurrent disease. In the ensuing 25 years, changes in staging systems, and clarification of prognostic factors have occurred, however, patients with early-stage RCC continue to relapse. Cumulative 5-yr survival rates following nephrectomy and lymphadenectomy range from 47% to 100% in stage $T_2N_0M_0$ to from 34% to 51% in patients with stage $T_3N_0M_0$ *(2)*. When regional lymph nodes are involved, five survival rates are even lower, and range from 6% to 43% *(2)*. Nodal status at surgery is often difficult to define, for reasons such as node matting or performance of lymph node sampling only instead of lymphadenectomy.

Patterns of recurrence in patients following resection of RCC have recently been reviewed *(3)*. One hundred and seventy-two patients undergoing nephrectomy were identified, and the majority ($N = 162$) had N_o disease. Distant metastases developed in 26%, and local recurrence in 5%. In this latter group, four of six patients also had distant metastases. Positive lymph-node status and renal-vein extension were independent prognostic factors associated with recurrence.

Findings such as these suggest patients with resected renal tumors are appropriate candidates for adjuvant therapy trials. Patients with stages II or III are at highest risk of

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Table 1
Adjuvant Therapy Employed in Patients Following Resection of Renal Cell Carcinoma

- Progestational Agents
- Radiotherapy
- Cytokines
- Chemotherapy
- Vaccines

Table 2
Adjuvant Trial Medroxyprogesterone Acetate (MPA) Following Radical Nephrectomy in Patients with Renal Cell Carcinoma

	Treatment Group	
	Observation	*MPA*
No. Patients:		
Entered	70	66
Evaluated	62	58
Stages:		
I	29	29
II and III	33	29
% Relapses	33.9%	32.7%
Median Time to Recurrence	11.0 mo	20.0 mo
5-yr DF Survival	67.3%	67.1%

[a] Pizzocaro et al. *(8)*.
DF—disease-free.

recurrence and have been included in the majority of adjuvant studies. The types of investigations previously conducted are outlined in Table 1. The patterns of recurrence in patients with RCC neoplasm suggest systemic approaches are the most appropriate.

2. MEDROXYPROGESTERONE ACETATE (MPA)

In 1971, Bloom *(4)* reviewed his experience with hormonal therapy of metastatic RCC in 80 patients (79 received MPA, 1 Delalutin). Eleven patients demonstrated improvement in radiologic or clinical signs associated with their tumors. Subsequent reports suggested hormonal therapy for metastatic RCC produces responses in 15 to 20% of cases. Recent data from randomized trials in which tamoxifen *(5)* or medroxyprogesterone *(6)* have been used as reference arms have not confirmed this level of activity. Uncontrolled trials suggested adjuvant MPA could decrease relapse rates compared to nonrandomized controls *(7)*. In view of this, prospective studies were undertaken.

Pizzocaro et al. *(8)* conducted a randomized controlled trial comparing 500 mg of MPA TIW for 1 yr following surgery with no additional treatment in patients following radical nephrectomy. Individuals with Robson stages I, II, and III were eligible. One hundred and thirty-six patients were entered, and 120 were evaluable. Results are summarized in Table 2, and demonstrate no differences in relapse rates, time to recurrence, or survival. The authors concluded that the toxicity of MPA and the absence of effects in this trial indicate it should not be utilized in the adjuvant setting.

3. RADIATION THERAPY

Radiotherapy has been used as an adjuvant to nephrectomy since the 1940s *(9)*. A series of historically controlled studies were published in the 1950s and 1960s, which suggested this modality improved survival in RCC patients *(10,11)*. In the 1970s, a series of randomized and controlled trials were initiated to evaluate the effects of adjuvant radiation. Recent reviews *(3,12)* of failure sites in patients undergoing nephrectomy suggests local failure is uncommon, and therefore modalities like radiation therapy for control local disease are unlikely to be of value. The results of the trials outlined in Table 3 are consistent with this conclusion.

Retrospective reviews in which patients undergoing nephrectomy were treated with postoperative irradiation suggested 5- and 10-yr survival rates could be improved at least 10% *(17,18)*. The prospective trials reported by Kjaer et al. *(15)* and Finney *(16)* could not confirm these reports. In the latter study *(15)*, significant postradiation complications developed in 44% of patients. The size of these trials (69 and 101 patients) probably precludes detection of the anticipated differences. Additionally, in the trial reported by Finney *(16)*, 40% of patients had clinical stage I tumors, a group in whom recurrence rates are expected to be low.

Preoperative radiation therapy for RCC was reported to produce a decrease in tumor size, fibrosis, decreased vascularization, and loss of proliferative capacity *(19,20)*. The two clinical trials *(13,14)* utilizing preoperative radiation therapy are also summarized in Table 3. No differences in recurrence rates or survival were found. In both studies significant numbers of patients with T_1 tumors were included. The authors also analyzed their findings by tumor stage, and found no effect in any category. As in the postoperative trials, the number of patients included was limited. The studies available therefore do not demonstrate an effect of pre-or postoperative adjuvant radiation therapy in RCC patients undergoing nephrectomy.

4. AUTOLOGOUS TUMOR VACCINES

Active specific immunotherapy (ASI) employing autologous tumor preparations with/without various adjuvants have been utilized to immunize RCC patients in the postoperative setting. In patients with metastatic disease, this approach has been associated with clinical tumor regression in selected patients *(21)*. Two adjuvant studies have been reported *(22,23)*, with one trial being historically controlled *(22)*, and the other, a prospective randomized study *(23)*. Repmann et al. *(22)* treated 162 patients (116 evaluable) with various stages ($T_{2\text{-}4}\ N_oM_o$,$T_{1\text{-}4}\ N_{1\text{-}3}\ M_o$, $T_{1\text{-}4}\ N_{0\text{-}3}\ M_1$). Results were compared to a matched group of 106 patients. The vaccine preparation (Macrophasm GmbH, Hannover) was described as an autologous tumor cell lysate "incubated with interferon-gamma and tocopherol acetate" "followed by washing steps to remove "lymphokine." When compared to the concurrent nonrandomized control group of 106 patients, a significant improvement in survival ($p = 0.0007$) was found. Two-year survival probability was 92.2% in the vaccine group compared to 75% in the control group. Analysis of various stages demonstrated no differences for Robson stages I and IV, whereas in patients with stages II and III two survival probability remained significantly improved in the treatment group. Toxicity of the vaccine preparation was reported as minimal.

Galligioni et al. *(23)* have reported a randomized prospectively controlled trial utilizing ASI following nephrectomy. Patients were randomized to observation or three

Table 3
Clinical Results of Adjuvant Radiotherapy Trials in Renal Cell Carcinoma Patients

Author (s)	*Therapy Arms*	*No. Patients*	*Stage Distribution*			*5-Year Survival (Percent)*	*Comments*
I. Preoperative Trials:			P1	P2	P3		
Van der Werf-Messing *(13)*	30 Gy in 3 wk	64	22	12	30	59%[a]	No differences in recurrence rates or survival.
	Surgery	62	21	19	22	64%[a]	
Juusela et al. *(14)*	30–36 Gy in 3 wk	38	8	12	18	47 ± 9%	No differences in 5-yr survival rates.
	Surgery	50	14	20	16	63 ± 7%	
II. Postoperative Trials:			I	II	III		
Kjaer et al. *(15)*	50 Gy on 20 fractions	32	-	17	15	50%[b]	No differences in survival/unacceptable toxicity.
	Surgery	33	-	17	16	62%[b]	
Finney *(16)*	55 Gy 27 fractions in 5˚ wk	52	23	9	19	36%	No influence on local recurrence or distant mets.
	Surgery	49	20	11	18	47%	

[a] Overall survival %.
[b] 2-yr survival rates.
P1, P2, P3—pathologic stages.
I, II, III—Robson stage.

intradermal injections of 10^7 autologous irradiated tumor cells mixed with 10^7 Bacillus Calmette-Guerin (first two injections). Patients with resected RCC TNM stages I, II, or III were eligible. One hundred and eighty-four patients were evaluated, and 120 were entered. Sixty-four patients were not randomized for reasons including insufficient cells for vaccination (24 patients) locally advanced and/or distant metastases (32 patients), and incomplete resection (three patients). At a median followup of 61 mo, the 5-yr disease-free survival rates were 63% in vaccine patients and 72% in controls. The overall survival rates were 69% and 78%, respectively. These results were not significantly different. The sample size employed was capable of detecting a 20 to 25% difference in disease-free survival. Results to date, therefore, do not demonstrate an adjuvant effect of ASI in patients with resected RCC. It is possible that differences in BCG strains and/or methodology tumor cell preparation may account for differences in these results compared to those reported in patients with colon cancer *(24)*.

5. CYTOKINE ADJUVANT TRIALS

A variety of cytokines have been utilized to treat metastatic RCC, with interferon alpha (IFNα) and interleukin (IL-2) having modest effects in this patient population. Recent reviews suggest IFNα produces a response rate of 10–15% *(25)* and a 2.5-mo improvement in overall survival compared to a control population *(6)* in patients with metastatic disease. Investigation of IFNα as an adjuvant following nephrectomy was therefore reasonable. Cockerell et al. *(26)* administered IFNα at a dose of 3 million units (MU) subcutaneously 3 d prior to surgery and for 14 d postsurgery to a group of 13 RCC patients with minimal metastatic disease. No major toxicity was encountered and the authors concluded IFNα was safe enough to be tested in larger studies. Takahashi et al. *(27)* treated 20 patients with stages I to III with IFNα2b in the postoperative period. Three MU intramuscularly for 28 d followed by 6 MU weekly for 12 mo was administered. Three patients developed recurrent disease, and significant increases in NK activity were noted in the treated patients. The authors suggest IFNα should be administered for a minimum of 5–7 mo when used as an adjuvant nephrectomy following in view of the delayed antitumor effects previously reported *(28)*.

These trials demonstrated the feasibility of postoperative IFNα administration. Three randomized studies were then performed to further evaluate the effects of this cytokine (Table 4). They *(29–31)* have been reported in preliminary form, and no improvements in disease free or overall survival for interferon treated patients was noted.

The studies differ in the types of interferons utilized (L-IFN, IFNα2a, IFNα2b), doses utilized, and duration of therapy (6 to 12 mo). Trump et al. *(30)* utilized L-IFN (lymphoblastoid IFN) in escalating doses (3 to 20 μ/m^2). A total of 266 eligible patients were entered, with 132 receiving L- IFN. At a median follow-up of 4.4 yr, recurrent disease developed in 58 control and 65 L-IFN treated patients ($p = 0.14$), with 37 and 54 deaths in the respective groups ($p = 0.02$). The investigators concluded L-IFN was not an effective surgical adjuvant.

The other two trials have utilized recombinant IFN preparations. Pizzocaro et al. *(31)* employed IFNα2b (6 MU TIW for 6 mo). A group of 269 eligible patients were entered with 134 receiving IFNα2b. Relapses were seen in 34% of IFN treated and 27% of control patients. In contrast, Porzsolt et al. *(29)* utilized IFNα2a (9MU TIW) for 12 mo a total of 270 patients with stages $T_{3\text{-}4}\ N_{o/+}\ M_{or}$ were randomized. One hundred and thirty-three

Table 4
Prospective Trials of Interferon on Renal Cell Cancer Patients Following Nephrectomy

Author(s)	*Stages Eligible*	*Treatment Groups*	*No. Patients*	*Comments*
Porzsolt et al. *(29)*	p T_{3-4}, p $N_{0/+}$, M_0	rIFNa2a-9MU SC TIW x 12 mo	133	No differences in time to treatment failure or survival.
		Surgery	137	
Trump et al. *(30)*	p T_{3-4a}, pN_{1-3}, M_0	L-IFN-3 to 20 MU/m^2 q 21d for 12 cycles	132	65 recurrences and 54 deaths in L-IFN arm compared to 58 recurrences and 37 deaths in surgery arm. L-IFN not an effective adjuvanta.
		Surgery	134	
Pizzocaro et al. *(31)*	Robson Stages II and III	rIFNa2b-6MU SC TIW for 6 mo	134	Preliminary results suggest no benefit from adjuvant IFNa.
		Surgery	135	

patients received IFNα. No differences in disease- free or overall survival between the two groups were found.

Three randomized trials utilizing intermediate doses of interferon have not demonstrated improvement in disease-free or overall survival with adjuvant administration. Additional studies investigating longer treatment durations (2 yr) or earlier stage patients (T_{1-2} or N_1) may be of interest. Additionally, use of high-dose induction IFNα regimens similar to those utilized in patients with malignant melanoma *(32)* is also an interesting alternative.

A second cytokine utilized as treatment for patients with metastatic RCC is rIL-2. Reviews *(33)* indicated objective responses are seen in 15% of patients receiving rIL-2, with a subset of patients having durable remissions. Studies utilizing rIL-2 in the adjuvant setting are now underway. Table 5 outlines these trials. The study reported by Olencki et al. *(34)* employed outpatient sc administration of rIL-2. Six to eight patients were treated with one of four different regimens for 6 mo to determine toxicity and tolerance. Preliminary results suggest 4.0 MIU/m^2 of rIL-2 can be administered subcutaneously for 6 mo post-nephrectomy with acceptable toxicity. In the first 15 patients treated, four have relapsed. A randomized trial utilizing this type of schedule is required.

The Cytokine Working Group (personal communication, 1999) is conducting a randomized trial in which high-dose iv rIL-2 is being utilized as an adjuvant following nephrectomy. Use of the high-dose regimen restricts patient entry, and the planned sample size ($N = 100$) in this trial is limited. The antitumor effects of rIL-2 previously reported suggest it should be evaluated as adjuvant therapy in RCC patients.

6. COMBINATION REGIMENS

The use of combination regimen such as rIL-2 and IFNα or chemoimmunotherapy employing this combination and 5-FU are of interest in view of the increased response rates as associated with these approaches *(33,35)*. Toxicity is also increased, and therefore may limit their utility in the adjuvant setting.

Migliari et al. *(36)* have conducted a pilot study utilizing IFNα and vinblastine in patients following nephrectomy and lymphadenectomy. Thirty patients with T_{2-3} N_o M_o RCC were treated. rIFNα2a was administered intramuscularly three times weekly in gradually increasing doses (from 3.0 to 18.0 MU over 4 wk) and vinblastine (iv bolus 0.1 mg/kg) every 3 wk for a total of four cycles. Moderate toxicity was reported, and 4/30 patients discontinued therapy. The results were contrasted with a historical cohort of 32 patients. Five-yr survival rates in the two groups were 83% and 50%, respectively ($p = 0.003$). The investigators suggest additional trials with this approach should be considered. Recent reports utilizing IFNα and vinblastine in patients with metastatic disease, however, do not suggest this combination is superior to IFNα alone *(37)*. Therefore, the rationale for further trials with this combination are unclear.

7. CONCLUSION

A series of adjuvant studies have been conducted in patients with localized RCC following nephrectomy. Currently, randomized studies have not demonstrated an advantage for pre- or postoperative therapy. Patient selection criteria have varied, but generally high risk group with T_{2-4} tumors and N_{1-3} disease have been included. Future studies investigating single agent IL-2, combination regimens, and chemoimmunotherapy will be of interest.

Table 5
Clinical Trials Employing rIL-2 as an Adjuvant in Renal Cancer Patients

Author(s)	*Dose & Schedule rIL-2*	*Type Trial*	*Patient Characteristic*		
			Stages	*Number*	*Comments*
Olencki et al. *(34)*	a) Doses: 2.0 or 4.0 MIU/m^2 sc BID b) Schedules: day 1–5 either qo wk or wk 1–4, 9–12, etc. for 6 mo	Pilot	a) $T_{3\text{-}4}$ N_o M_o b) T_{any} $N_{1\text{-}3}$ M_o c) T_{any} N_{any} M_{oR}	30	Preliminary trial to demonstrate tolerance to various rIL-2 regimens nephrectomy.
Cytokine Working Group *(35)*	600,000 IU/kg IV q 8 h d 1–5 and 15–19	Phase 3	a) $T_{3c\text{-}4}$, N_{01} b) T_{any}, $N_{2\text{-}3}$ c) T_{any} N_{any} M_{or}	100	Trial in progress.

M_{or} —metastatic disease, resected.
sc — subcutaneous.
ivB — intravenous bolus.

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III Management of Advanced and/or Metastatic Renal Cell Carcinoma

14 The Role of Nephrectomy and Metastasectomy for Advanced Renal Cell Carcinoma

Robert G. Uzzo and Andrew C. Novick

CONTENTS

1. INTRODUCTION

Renal cell carcinoma (RCC) accounts for over 85% of all solid kidney tumors and is the third most common genitourinary malignancy after carcinoma of the prostate and bladder. Approximately 28–33,000 new cases of RCC are diagnosed per year in the United States, accounting for 3% of all adult malignancies, with an incidence roughly equal to that of all forms of leukemia combined *(1)*. Over one third of patients with RCC will ultimately die of their disease. Despite the increase in incidentally discovered tumors due to the widespread use of noninvasive radiographic imaging techniques *(2–4)*, 25–30% of patients present with locally advanced or metastatic disease *(5)*. Delay in diagnosis may result from the ability of space occupying lesions of the retroperitoneum to become quite large before causing local symptoms. Additionally, manifestations of RCC are protean and may give rise to a constellation of nonspecific symptoms causing delayed detection or discovery of the lesion while pursuing other diagnoses. Finally, microscopic hematuria may go undetected while gross hematuria, which may occur only after the primary lesion has reached considerable size, may be improperly misattributed to other causes.

The role of surgery in patients with advanced and metastatic RCC remains the subject of some debate and its use has been influenced by a number of factors. Current operative and perioperative care is associated with low morbidity and mortality because nephrectomy often involves minimal functional sacrifice if the contralateral kidney is normal.

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Table 1
Proposed Indications for Nephrectomy in Cases of Advanced Renal Cell Carcinoma

1. Therapeutic:
   Adjunctive nephrectomy to improve overall patient survival. Strategies include:
   a. unusual association between nephrectomy and spontaneous regression;
   b. resection of primary and metastatic foci (simultaneous or staged) to render patient no evidence of disease (N.E.D.);
   c. cytoreductive surgery followed by adjuvant therapy;
   d. nephrectomy as part of an adoptive immunotherapy protocol.
2. Symptomatic:
   To control manifestations of advanced disease and improve quality of life:
   a. alleviation of or prophylaxis against local manifestations by palliative nephrectomy or angioinfarction;
   b. control of systemic or paraneoplastic manifestations;
   c. improvement of emotional or psychological stress associated with the disease.

In addition, the disease typically affects young, otherwise healthy and often assymptomatic individuals, so there may be overwhelming emotional pressure placed on the physician by the patient and his or her family to intervene. These factors taken with the lack of any consistently effective adjuvant systemic therapy create a sense of urgency for patients newly diagnosed with advanced stage disease. Finally, the biological behavior of RCC is somewhat less predictable than other solid malignancies, which may reflect its well documented but variable responsiveness to antitumor immune strategies. Overall, these issues help maintain interest by patients and physicians alike in pursuing aggressive surgical options for selected individuals with advanced RCC.

The proposed indications for surgical intervention in cases of advanced or metastatic RCC can be summarized readily (Table 1). In the setting of advanced disease, nephrectomy or metastasectomy should be undertaken in two distinct circumstances; to achieve a therapeutic benefit and improve the patient's overall survival, or to control symptomatic manifestations of advanced disease. Inherent to attaining these goals is a thorough knowledge of the limitations of the existing data, placed in the clinical context of the individual patient's circumstances with consideration to issues relating to quality of life. In this chapter, the natural history of metastatic RCC and the risks associated with surgery will be reviewed briefly to provide the appropriate context for a meaningful examination of the existing data regarding the role of nephrectomy and/or metastasectomy in patients with advanced RCC.

2. NATURAL HISTORY OF RENAL CELL CARCINOMA (RCC)

The natural history of RCC refers to its progression in the untreated patient and is therefore difficult to quantitate accurately. Most RCCs arise from the proximal tubular epithelium and grow in a local rather than infiltrating pattern. Therefore, unlike carcinoma of the prostate, RCC develops as discrete, focal, mass lesions. Local extension through the renal capsule and beyond Gerota's fascia may lead to involvement of contiguous retro- or intraperitoneal structures such as psoas, diaphragm, liver, bowel, or mesentery. Invasion into segmental branches of the renal vein is not uncommon. Subsequent extension into the main renal vein and inferior vena cava (IVC) allows hematog-

Table 2
Survival of Patients Presenting with Metastatic RCC

		% Survival	
Reference	*n*	*1 year*	*5 year*
Middleton *(50)*, 1967	141	7	0
Bottiger *(74)*, 1970	40	42	4
Rafla *(75)*, 1970	24	13	0
Thompson *(76)*, 1975	65	22	0
Klugo *(77)*, 1977	101	12-52[a]	0-14[a]
deKernion *(54)*, 1978	41	10	0
Selli *(31)*, 1983	18	34	–
Giuliani *(78)*, 1990	50	–	<7

[a] Varied with mode of therapy. Revised and adapted from Fowler *(27)*.

enous spread to the lungs, bones, and central nervous system (CNS). Metastases have been described in virtually every part of the body including the tongue *(6)*, tonsils *(7)*, thyroid *(8,9)*, eye *(10,11)*, breast *(12,13)*, gallbladder *(14,15)*, pancreas *(16)*, stomach *(17)*, heart *(18)*, spermatic cord*(19)*, seminal vesicle *(20)*, bladder *(21)*, vagina *(22)*, penis *(19)*, and a myriad of skeletal muscle *(23)* and soft tissue sites *(24,25)*. Symptomatic manifestations of metastatic or recurrent RCC are therefore quite variable. RCCs can also metastasize through lymphatic channels to regional and mediastinal nodes. Due to variable lymphatic drainage in the upper abdomen, nodal metastases in RCC do not always follow a predictable pattern thereby limiting the utility of therapeutic lymphadenectomy *(26)*.

In general, the incidence of metastases increases with the size of the primary lesion *(27)*. Despite this tendency, metastases may occur from very small lesions, and conversely the primary lesion may become quite large in the absence of identifiable extrarenal disease. Symptoms may be due to either local involvement of the tumor causing pain, hematuria or a flank mass, or from manifestations of metastatic disease such as fever, weight loss, or other constitutional symptoms. Paraneoplastic syndromes (*see* Chapter 8) occur in as many as 30% of patients with RCC and may include hypercalcemia, hypertension, pyrexia, erythrocytosis, and hepatic dysfunction *(28)*. As a rule, existing metastases progress and new lesions appear until ultimately, cachexia, pain, persistent hematuria, venous thrombosis, pulmonary emboli, and other terminal manifestations arise in the setting of diffuse disease. The majority of patients presenting with metastatic disease die within one year of diagnosis because of complications arising from their metastases *(27)* (Table 2).

Confounding the management of patients with advanced disease is the unusual and unpredictable tendency of some lesions to remain quiescent or in rare instances regress. The growth rate of individual metastases is not always uniform and specific lesions may remain stable whereas others expand. This may result in the unexpected survival of patients with metastatic disease for long periods. The literature is replete with studies evaluating prognostic factors (*see* Chapter 9) associated with progression in an attempt to identify the mechanisms responsible for this unusual deviation from the expected natural history of advanced RCC. The most relevant and well supported of these factors include patient characteristics such as extent of symptoms at presentation, performance

status and disease free interval, as well as tumor characteristics such as pathologic stage, histology and grade *(29)*. Published reports assessing competing strategies for the management of advanced RCC must be evaluated based on their ability to control for these variables.

As our understanding of the natural history of RCC has improved, the short- and long-term risks associated with surgical management of the disease have continued to decline. Standard nephrectomy is typically associated with a brief hospital stay and several weeks of postoperative convalescence. Laparoscopic nephrectomy may decrease in-patient care and morbidity even further *(30)*. Improvements in monitoring and acute care continue to reduce the risks of nephrectomy such that significant post operative complications occur in less than 10% of patients, with perioperative mortality rates between 0 and 5% *(27)*. The risk is somewhat higher for patients with advanced and metastatic disease because of general patient debility, larger tumor burden, and local or venous involvement *(31)*. In the absence of randomized clinical trials, the dismal natural history of metastatic RCC, combined with our ability to surgically remove the lesion(s) successfully and with minimal morbidity, not only influences our treatment strategies, but also generates the selection bias inherent to most studies on this subject.

3. THERAPEUTIC NEPHRECTOMY FOR ADVANCED DISEASE

The rationale for therapeutic nephrectomy in patients with advanced (M+) RCC can be separated into four proposed mechanisms of benefit. In the first, there is a small body of literature that demonstrates spontaneous regression of metastatic sites upon removal of the primary tumor. Second, nephrectomy in the setting of metastatic disease may be combined with metastasectomy, either simultaneously or as a staged procedure, in an effort to surgically remove all viable tumors and render the patient with no evidence of disease (N.E.D.). The third therapeutic indication for nephrectomy in patients with disseminated disease is to reduce the overall tumor burden, thereby making adjuvant systemic therapies more effective. This is commonly referred to as cytoreductive nephrectomy. Finally, the primary lesion can be removed to obtain tumor antigen for adoptive immunotherapy protocols. The data for each of these indications will be reviewed separately.

3.1. Induction of Spontaneous Regression

Spontaneous regression of RCC is a rare and intriguing phenomenon that has been reported in cases of both primary and metastatic lesions in response to nephrectomy, radiation, angioinfarction, and even during periods of observation. Over 100 cases have been reported in more than 35 peer-reviewed manuscripts published on spontaneous regression of metastatic RCC, most of which are isolated case reports or small series. Unfortunately, many of the reported cases are retrospective and not histologically confirmed. In addition, because the total number of patients with metastatic RCC, the denominator, is rarely reported in these articles, the true incidence of spontaneous regression is unknown. Everson and Cole were the first to systematically collect cases of spontaneous regression of cancer. In their initial report, they identified a total of 176 cases of possible spontaneous tumor regression, of which RCC was the most common visceral malignancy representing 18% of the total *(32)*. Accounts in the literature estimate the incidence of spontaneous remission to be as low as 0% and as high as 24%. In a review of 533 con-

secutive patients with advanced RCC followed at the Mayo Clinic, Myers found no cases of spontaneous regression *(33)*. In contrast, Oliver reported spontaneous regression of distant lesions in 5 of 69 patients (7%) with metastatic RCC followed prospectively *(34)*. Others have noted incidences as high as 24%, including nonprogressing lesions *(35)*.

Reported regression of metastatic sites includes those to brain *(36,37)*, bone *(38)*, hilar adenopathy *(39)*, liver *(40)*, caval thrombus *(41)*, and most commonly pulmonary metastases *(37,42)*. Few cases have been documented histologically thereby making these findings more questionable, particularly in cases of pulmonary nodules where sarcoidosis, infarcts *(43)* and fungal disease may coexist in patients with disseminated RCC, all of which can mimic a metastatic nodule and are known to regress spontaneously. Many of these cases report complete disappearance of disease, but with variable duration of follow-up. In fact, most tumor regressions appear to be short lived (12–24 months) and are not clearly associated with prolongation of survival. Unfortunately, in these instances, recurrent and progressive disease is generally the rule *(44)*.

The etiology of spontaneous regression or stabilization of disease is unclear. Most authors have attributed this phenomenon to immunologic host factors and used the occurrence of spontaneous regressions as indirect proof that endogenous host antitumor immunity can occasionally be effective against tumor progression. Few studies have attempted to correlate spontaneous remission with specific cell mediated antitumor immune parameters. Abubaker could document no differences in cytotoxic effector cell activity or proliferation including lymphokine activated killer (LAK) cells, natural killer (NK) cells, and mixed lymphocyte reactions in a patient with spontaneous regression of a histologically confirmed mediastinal RCC mass *(45)*. The true mechanism of these rare spontaneous regressions and its relationship to antitumor immunity therefore remains unclear.

The impact of the primary lesion on metastatic sites has been the subject of some speculation. The primary lesion has been shown to produces a number of metabolically active substances that may promote tumor growth by diminishing the T-cell response against autologous tumor antigen *(46,47)*. In contrast, other investigators have found that primary tumors may produce factors that inhibit distant tumor growth such as the potent antiangiogenic substance angiostatin *(48)*; however, this has not yet been demonstrated in RCC. Contradictory anecdotes of spontaneous tumor regression of distant lesions following nephrectomy versus rapid growth of metastatic sites after removal of the primary lesion underscore the poorly understood relationship between the primary tumor and its metastases. Currently, it appears the potential morbidity associated with nephrectomy in the setting of extensive disease exceeds the remote possibility of inducing a spontaneous regression. This phenomenon should, therefore, be considered a clinical curiosity and an impetus for research regarding the interaction between the tumor and host immune defenses rather than an indication to operate.

3.2. Complete Tumor Resection to Render the Patient No Evidence of Disease (N.E.D.)

Evaluation of the role of complete tumor resection in patients with metastatic RCC and its effect on prolongation of survival is hampered by a lack of prospective randomized clinical trials and outcome data examining the issue. The wide spectrum of clinical scenarios and inherent selection bias reported in the literature makes generalizations on this topic difficult. Clearly, the best patient for resection of metastatic disease is one with

a high performance status and a solitary, metachronous, pulmonary lesion that presents after a long (>3 year) disease free interval. Conversely, the worst candidate is one with a poor performance status and multiple synchronous metastases. The former patient has a >50% chance of surviving an additional two years *(49)* (Table 3), whereas the mean survival of the latter patient is four to ten months *(27)* (Table 2). This example underscores the confounding effect of patient selection in evaluating the role of surgery for metastatic RCC and may reflect the natural history of the individual's disease more than the intervention itself. Aggressive surgical extirpation of lesions presenting in patients between these two clinical extremes is less certain and treatment decisions are often based on more subjective criteria than strict outcomes data.

Middleton was one of the first to report on the role of surgery for metastatic renal cell carcinoma *(50)*. He reported on 503 cases of RCC from the New York Hospital and noted that among 33 patients presenting with multiple synchronous metastatic lesions who underwent nephrectomy, 93% died within one year. Eight patients underwent complete surgical resection of solitary metastatic lesions, four at the time of their nephrectomy and four who represented with a solitary metastasis. Only those who had excision of a lesion presenting metachronously gained a survival advantage. Tolia and Whitmore reviewed the data on 174 patients with stage IV RCC treated at Memorial Sloan Kettering and noted that 32% (6/19) with synchronous, solitary metastasis survived five years after resection, and/or XRT; however, most patients experienced recurrence despite aggressive treatment and only one patient was alive at 10 years *(51)*. O'Dea reviewed the Mayo Clinic data on resection of solitary RCC metastasis and separated groups into those whose metastatic lesion presented synchronously versus those that presented at some time after the original nephrectomy. He found that 23% with metachronous solitary lesions lived more than five years after resection while only 22% of those whose solitary metastasis was resected at the time of nephrectomy survived more than two years *(52)*. Subsequent studies by Golimbu *(53)*, deKernion *(54)*, and Kavolius *(49)* corroborate the finding that favorable predictors of survival include a single, metachronous site of recurrence with a long disease-free interval.

Multiple studies confirm that the most favorable lesions for resection are solitary pulmonary masses (*see* Chapter 15). Cerfolio reviewed the data from 96 consecutive patients treated at the Mayo Clinic who underwent complete pulmonary resection for metastatic renal cell carcinoma and noted a 45.6% 5-yr survival rate for patients with solitary metachronous lung lesions *(55)*. Similarly, in a recent series from Memorial Sloan Kettering the five-year survival rate was 54% in 50 patients treated surgically for a solitary lung metastasis *(49)*. Improved survival in patients with pulmonary metastasis may be because of earlier detection and/or less associated functional impairment than with metastases to other organs such as the CNS *(56)*. It remains to be determined whether RCC clones capable of metastasizing to the lung are biologically different than those that metastasize elsewhere. Newly developed molecular tools such as transcript array analysis, which simultaneously screens thousands of genes for expression in selected tissues, may help discern if a difference exists in metastatic clones at various sites at the genetic level.

In summary, the three primary determinants of success for surgery in the setting of metastatic RCC include; timing of the metastatic presentation with metachronous lesions and long disease-free intervals faring best, the number and location of the lesions with

Table 3
Survival After Resection of Metastatic RCC

Reference	*n*	*Metastatic Site(s)*	*Solitary or Multiple*	*Synchronous or Metachronous*	*% Overall 5-Yr Survival*
Middleton *(50)*, 1967	4	brain/lung	solitary	metachronous	25
	4	brain/lung	solitary	synchronous	0
Tolia *(51)*, 1975	19	bone/lung/LN/soft tissue	solitary	synchronous	35
O'dea *(52)*, 1978	26	bone/brain/lung/LN	solitary	metachronous[a]	23
	18	bone/brain/adrenal	solitary	synchronous	6
Golimbu *(53)*, 1986	13[b]	bone/lung/fossa	solitary (6) multiple (7)	metachronous	25
	8[c]	bone/lung/adrenal/pancreas	solitary (4) multiple (4)	synchronous	50
Dineen *(79)*, 1987	18	bone/brain/lung/soft tissue/LN	solitary	metachronous[d]	13
	11	bone/brain/lung/soft tissue/LN	solitary	synchronous	13
Kiernery *(80)*, 1994	41	bone/brain/lung/soft tissue	solitary	metachronous[e]	31
Cerfolio *(55)*, 1994	48	lung	solitary	metachronous[f]	46
	48	lung	multiple	metachronous[f]	27
Kavolius *(49)*, 1998	155	bone/brain/lung/soft tissue/LN/fossa	solitary	metachronous[g]	54
	123	bone/brain/lung/soft tissue/LN/fossa	multiple	metachronous[g]	29

LN = lymph node.
[a] Mean disease-free interval of 34 months.
[b] Complete surgical excision in 6/13. Mean disease-free interval of 29 months.
[c] Complete surgical excision in 5/8 cases.
[d] Mean disease-free interval of 38 months.
[e] Median disease-free interval of 27 months.
[f] Includes four patients with synchronous lesions. Median disease-free interval of 41 months.
[g] Median disease-free interval of 25 months.

solitary pulmonary metastases being most responsive to surgical resection, and the patient's overall performance status *(29)*.The incidence of subclinical, multifocal, microembolic disease is unknown, but may explain early disease progression after aggressive surgery. A thorough clinical staging is mandatory before proceeding with any aggressive surgical resection. Whether the existing data on the topic is a true reflection of the surgical intervention or of selecting less biologically aggressive tumors for surgery remains to be fully answered.

3.3. Cytoreductive Nephrectomy

The role of nephrectomy as part of multimodality therapy consisting of surgery and immuno- or chemotherapy in patients with metastatic RCC continues to be assessed. Two different strategies have been suggested; in the first, nephrectomy is performed to debulk or cytoreduce the tumor burden in an attempt to make systemic therapy more effective; in the second, systemic therapy is administered up front to determine responsiveness and nephrectomy is performed in responders. Whereas the response rate to initial systemic therapy is quite low, many investigators and clinicians have taken the more aggressive approach of initial cytoreductive nephrectomy. In evaluating this treatment strategy for patients with metastatic RCC, two central issues must be addressed; what percentage of patients will go on to receive immunotherapy postoperatively, and does cytoreduction increase the likelihood of an objective response to immunotherapy?

Several large studies have addressed the role of cytoreductive nephrectomy and evaluated the number of patients who ultimately receive adjuvant systemic therapy. Perhaps the greatest criticism of this approach is that the morbidity of the surgery or interval disease progression may limit the number of patients who receive aggressive systemic therapy. Using strict selection criteria, Fallick reported 93% (26/28) of patients were able to receive systemic therapy after cytoreductive nephrectomy *(57)*. Investigators at UCLA demonstrated that 87% (48/55) went on to receive immunotherapy post operatively *(58)*, whereas 78% (29/37) could receive multimodality treatment after nephrectomy in a cohort of patients treated at the Cleveland Clinic Foundation *(59)*. In the largest series of its kind, Walther at the National Institute of Health recently reported on 195 patients treated over 11 yr with cytoreductive nephrectomy prior to receiving adjuvant high-dose interleukin 2 (IL-2) and noted only 55% (107/195) were able to received cytokine therapy *(60)*. Differences in selection criteria between studies such as these make direct comparisons difficult, whereas patient selection criteria varied between institutions and individual surgeons. Nonetheless, the primary determinants should include an assessment of the patient's performance status, cardiopulmonary reserve, the resectability of the primary lesion, and the sites and number of metastases to be removed. Overall, the literature would suggest that with appropriate selection criteria, surgical morbidity should not be a limiting factor in determining who should undergo debulking nephrectomy and/or metastasectomy.

The importance of reducing tumor burden to make systemic therapy more effective continues to be defined. The existing clinical data are difficult to summarize in this regard because of lack of prospective, randomized trials, differing immunotherapy protocols, and variable selection criteria. The most uniform experience exists with adjuvant IL-2. Most centers recognize an objective response rate of 15–20% with high-dose IL-2, including a 3–5% complete response rate *(60)*. However, the durability of response is variable and most patients receive additional biologic response modifiers, chemotherapy, or radiation as tolerated.

Emerging evidence suggests that the tumor itself may play an important role in evading host antitumor immune defenses. The tumor environment and/or associated stroma are known to produce a variety of immunosuppressive molecules. These products may facilitate tumor evasion of the immune system by blocking intracellular signaling and effector function of T lymphocytes. Hydrogen peroxide derived from tumor-associated macrophages may be involved in depressing TCRζ levels in lymphocytes from tumor bearing mice and cancer patients *(61)*. Prostaglandins (PGE2) *(62)*, IL-10 *(47,63)*, and tumor-derived glycosphingolipids *(64)* present within the tumor environment can all inhibit DNA binding of the transcription factor NFκB which is requisite for normal T-cell activation. Removal of all viable tumor has been shown to reverse this T-cell defect in a subset of patients *(46)*. Additionally, the tumor may also directly reduce T-cell-mediated activity by the induction of apoptosis in activated T cells via the FasL(CD95/CD95L) pathway *(65)*. As mechanisms of tumor-induced suppression of endogenous antitumor immunity continue to be elucidated, the role of cytoreductive nephrectomy may increase in the management of patients with advanced RCC.

3.4. Nephrectomy as Part of Adoptive Immunotherapy Protocol

Given the poor response rates to current systemic therapies for advanced RCC and the recognition that renal tumors are among the most sensitive solid tumors to immunotherapy strategies, investigators continue to design protocols to induce a more potent antitumor immune response. The basis of most T-cell adoptive immunotherapy techniques is the identification of a substantial, mixed lymphoid population in RCC tumor beds consisting of clones capable of preferentially recognizing tumor antigen as well as nonspecific cytotoxic T lymphocytes *(66,67)*. Tumor progression may result from inadequate effector function of these infiltrating T cells, a notion supported by the finding that within the tumor bed T-cell signaling is defective and there is little evidence for induction of a Th1 type cytokine response *(68)*. Understanding these defects and engineering a more specific functional T-cell response is central to any effective antitumor immune strategy. Nephrectomy in the setting of advanced RCC therefore provides TIL populations for basic studies as well as in vitro manipulation and subsequent readministration to the patients (*see* Chapters 21 and 23). Isolation of TILs and in vitro expansion is both costly and labor intensive and has met with only modest clinical success *(69)*; however, these studies continue to teach us a great deal about the host antitumor immune response. More recent strategies to enhance adoptive immunotherapy protocols include the use of genetically modified TILs, combination cytokine therapy, and in vitro activation of T cells from tumor-draining lymph nodes, which are believed to be sensitized to tumor specific antigens. Ex vivo manipulation of T cells from tumor-draining lymph nodes can reverse several T-cell signaling defects and may be associated with acquisition of a more potent antitumor activity *(70)*.

Nephrectomy also provides tumor specific antigen that can be used to prime the immune response as part of an adoptive immunotherapy strategy. Recent basic and clinical interest in dendritic cell protocols require autologous tumor to generate these highly specific antigen presenting cells. Initial studies demonstrate that functional dendritic cells (DC) can be generated from peripheral blood monocytes/macrophages in the presence of GM-CSF and IL-4. These DCs are then loaded with autologous tumor lysate, the antigens from which they present to T cells. Lymphocytes primed in this manner have been shown to be better mediators of autologous tumor lysis *(71)*. As tumor specific antigens become

better characterized, the role of these potent antigen presenting cells and our ability to manipulate them for therapeutic gain promises to increase.

Nephrectomy as part of an adoptive immunotherapy trial requires a systematic, dedicated team approach consisting of the urologic surgeon, a medical oncologist, and immunologist. These types of trials should be undertaken at dedicated academic centers where the ability to recruit large numbers of patients exists to provide sufficient power to the studies. Patients must also understand the risks, limitations and potential benefits of such clinical trials. Additionally, strict selection criteria must be established and objective, clinical, and biological measures of response should be sought in an effort to provide an appropriate appraisal of the role of nephrectomy for this indication.

4. PALLIATIVE NEPHRECTOMY IN PATIENTS WITH ADVANCED RCC

In rare instances, nephrectomy is indicated for alleviation of local or systemic manifestations of advanced RCC. In a series of 78 patients with advanced RCC treated at the Cleveland Clinic Foundation, Montie noted only 28% of patients had symptoms related to the primary tumor and these were not difficult to manage in patients who had not undergone nephrectomy *(72)*. Although, pain attributable to the primary tumor is experienced by as many as 40% of all patients with RCC *(27)*, in most instances, this can be relieved effectively with oral, transdermal, or parenteral agents. Local invasion of adjacent muscle or nerve roots may cause chronic, debilitating pain, that in rare instances, is unresponsive to pharmacological treatments. In such cases, nephrectomy is usually technically very difficult and often incomplete with poor survival. Significant hemorrhage or recurrent clot colic from locally advanced RCC is another unusual sequela of advanced disease. Anemia associated with metastatic disease is more often a manifestation of chronic disease than of urinary blood loss. In the very rare event of profuse life-threatening hematuria from spontaneous tumor rupture or symptomatic massive arteriovenous fistulae, the primary tumor can be successfully treated by angiographic embolization *(73)*. Palliative angioinfarction has the advantages of being less invasive, less morbid, less expensive, and can be performed as an outpatient on multiple symptomatic metastatic sites simultaneously. Finally, palliative embolization is usually well-tolerated regardless of the patient's performance status. Therefore, the role of nephrectomy for palliation or prophylaxis against local manifestations of bulky disease is truly limited. Additionally, despite the myriad of constitutional and paraneoplastic symptoms that may be attributable to advanced disease, there is little evidence to suggest that removal of the primary lesion will alleviate these manifestations.

5. CONCLUSIONS

The primary aim of the urologic surgeon involved in the management of patients with metastatic RCC should be to provide objective and realistic expectations regarding the role of surgery without eliminating the essential element of hope. Ultimately, the patient and his or her family must be intimately involved in the decision-making process. When considering the role of therapeutic nephrectomy, patient selection is of paramount importance. Patients with a high-performance status will invariably fare better. Additionally, the number of lesions, their location and the timing of the metastatic presentation are key

prognostic variables. Published reports on this topic should be evaluated based on their ability to control for these variables. Spontaneous regression of metastatic RCC should be considered an oncologic curiosity and its relationship to the status of the primary tumor is unproven. Resection of solitary metastases is of most benefit in patients with metachronous lesions and a long disease-free interval. Cytoreductive nephrectomy prior to immunotherapy is often technically feasible and most well-selected patients will go on to receive postoperative systemic therapy. However, in evaluating these types of studies, one must measure any benefit derived from this form of therapy against the inherent selection bias. Nephrectomy as a component of adoptive immunotherapy whose aim is to understand the complex interaction between tumor and host currently holds the most promise. Finally, our current understanding of treatment options and the therapeutic role of nephrectomy in patients with metastatic RCC is hindered by a lack of prospective randomized clinical trials and outcome analyses.

As physicians, a proactive approach to the treatment of this disease remains central to our way of thinking. Some form of therapeutic intervention is often expected and necessary to maintain the will of the patient, the family, and the treating physician. These pressures must be weighed against issues relating to quality of life in these terminally ill individuals and the ultimate need to advance our understanding and management of this disease.

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15 Management of Pulmonary Metastases in Renal Cell Carcinoma Patients

Thomas W. Rice

CONTENTS

1. INTRODUCTION

Barney and Churchill *(1)* were the first physicians to document the benefit of pulmonary metastasectomy in a patient with renal cell carcinoma (RCC). They performed a partial resection of the left upper lobe in a 55-year-old female 14 mo after nephrectomy and thoracic radiation for RCC with a synchronous pulmonary metastasis. The patient survived twenty-three years after surgery and ultimately died of coronary artery disease *(2,3)*. Initially, pulmonary metastasectomy was viewed a clinical curiosity and infrequently used in the treatment of lung metastases *(3,4)*. Over the last two decades, there has been a slow, but steady, increase in the use of pulmonary resections in highly selected patients with stage IV disease. Today, pulmonary metastasectomy is a viable option in the management of patients with stage IV RCC. Unfortunately, identifying which patients will most benefit from this aggressive surgical approach is a major problem because the tumor and host factors that allow systemic disease to be controlled with local therapy are unknown.

2. PATHOLOGY AND EPIDEMIOLOGY

Formation of metastases from a primary tumor requires angiogenesis, vascular or lymphatic invasion, transportation of malignant cells, evasion of host defenses, implantation,

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and proliferation. At the site of metasasis, this process repeats and allows metastases to metastasize (cascade metastases). In most patients with RCC, pulmonary metastases are the result of hematogenous dissemination from the primary tumor. It is estimated that 6% to 8% of all pulmonary metastases appear as lymphatic disease *(5)*. Lymphatic transportation of malignant cells from the abdominal lymphatics to mediastinal and hilar lymphatics, followed by retrograde invasion of pulmonary lymphatics can produce pulmonary metasatses. It is, however, lymphatic spread from a pulmonary metastases that cause most pulmonary lymphatic metastases *(6)*. Retrograde lymphatic extension into pleural lymphatics will result in pleural metastases. Pulmonary metastases may also arise from other distant metastases. Endobronchial metastases may occur by direct invasion from parenchymal, mediastinal or lymphatic metastases, systemic tumor emboli into the bronchial arteries, or aspiration of tumor present in the upper aerodigestive tract.

The most common site of metastatic RCC is the lung. Pulmonary metastases can be in as many as three-quarters of patients with stage IV RCC *(7–9)*. The next most common sites are liver and bone. At diagnosis of the primary RCC, 25% to 40% of patients will have metastases *(9,10)*. Up to one-half of patients undergoing nephrectomy for clinically isolated disease will develop metastases *(10)* and in 70%, metastatic disease will be detected within the first year *(11)*. Reportedly metastases confined to the lung have a survival advantage when compared to other metastatic sites *(7,10)*.

3. DIAGNOSIS AND SCREENING

Because the majority of pulmonary metastases are peripheral, 10% to 15% of patients are symptomatic. Many renal cell pulmonary metastases are discovered on routine chest X-rays (Fig. 1), however, chest X-rays are not sensitive in screening for pulmonary metastases *(12)*. Although a PA and lateral chest X-ray is usually included in the evaluation prior to resection of a RCC, it is best to add a CT examination of the chest (Fig. 2). Follow-up of a patient with a resected RCC should include a history, physical examination, CT abdomen, and CT chest. Because most metastases are discovered soon after nephrectomy, screening examinations should be scheduled every 3 to 6 mo in the first 2 to 3 yr following resection (Fig. 3). The follow-up period can be extended to once every 6–12 mo after this interval. Following the resections of a RCC and pulmonary metastases, a CT of the abdomen and chest should be routine in an increased surveillance protocol.

In patients with RCC, not all pulmonary nodules are metastatic. Approximately 50% to 70% of solitary pulmonary nodules will be metastatic; others are primary bronchogenic carcinomas or benign nodules *(13,14)*. A short disease-free interval (0–39 mo) is common in patients with pulmonary metastases from RCC, whereas a longer disease-free interval (48–51 mo) is common in patients with metachronous pulmonary primary carcinomas *(14)*. Multiple pulmonary nodules are most frequently malignant and metastatic, with 16% of these being benign *(15)*. Not all nodules resected in a patient with multiple pulmonary metastases are malignant, a significant proportion are either benign or secondary to previous systemic therapy.

4. PATIENT SELECTION

Surgery is the only potentially curative therapy for patients with pulmonary metastases from RCC, but only if all metastatic disease can be resected and the patient can

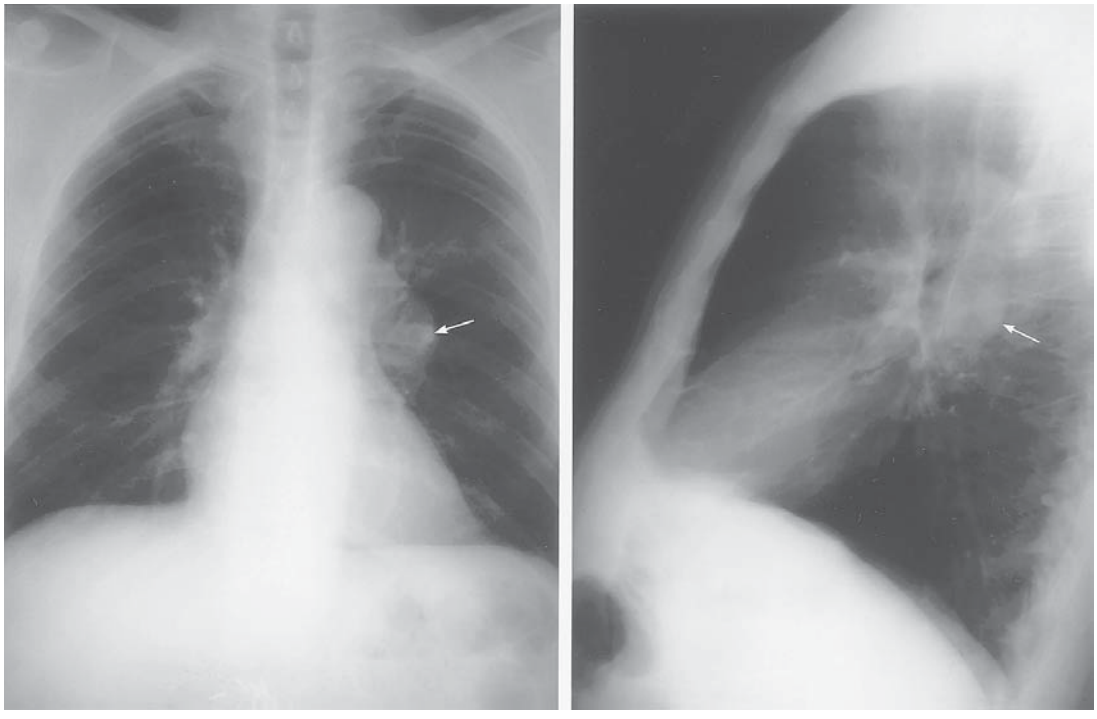

Fig. 1. PA and lateral chest film of a patient following right nephrectomy for RCC. The PA and lateral film show a hilar mass [arrows].

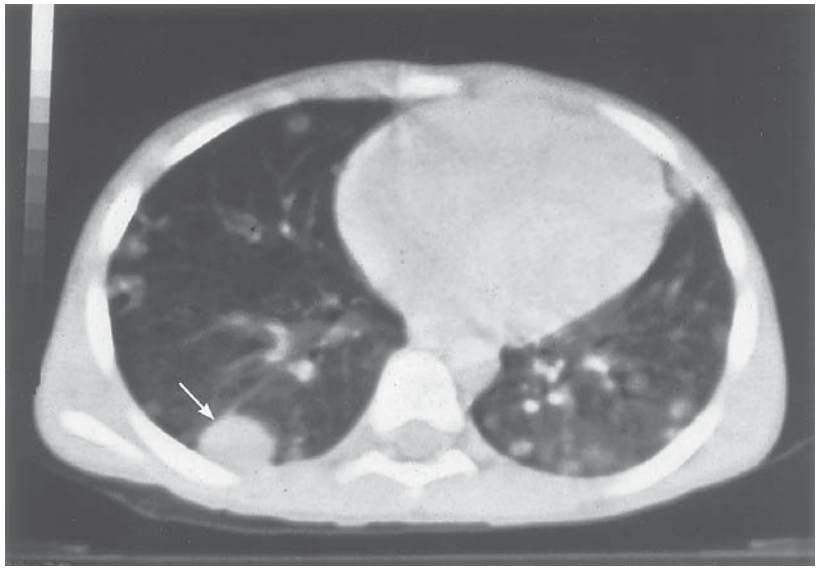

Fig. 2. In a patient undergoing evaluation for nephrectomy, a preoperative chest X-ray demonstrated a right lower-lobe lung lesion. Chest CT scan demonstrates the dominate right lower-lobe pulmonary nodule [arrow] with multiple bilateral metastases.

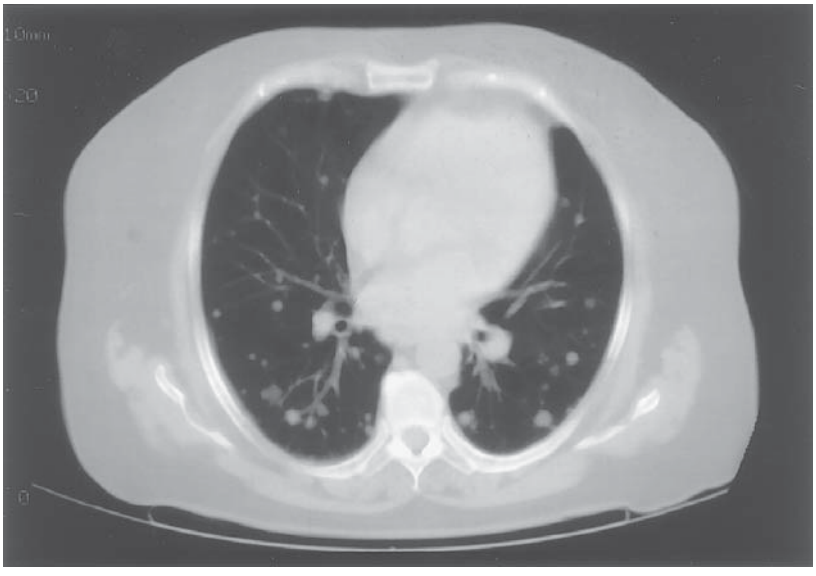

Fig. 3. Follow-up chest CT scan in a patient following nephrectomy for RCC. The scan demonstrates innumerable pulmonary metastases. The patient is not a candidate for pulmonary metastasectomy.

survive the operation. Criteria for patient selection are (1) the primary RCC is controlled or controllable; (2) other sites of distant spread are spared and the lungs are the only site of metastases; (3) all pulmonary metastases are resectable; (4) the patient's pulmonary status is adequate for the planned resection; and (5) no major comorbidity is present.

Determinates for long-term survival following resection of pulmonary metastases are the ability to completely resect all metastatic disease, a single metastatic focus, and a disease-free interval of 36 mo or more *(16)*. In small, retrospective studies there is no unanimity for the factors associated with curative resection of pulmonary metastases of renal origin *(17–24)*. Age, gender, and primary site within the kidney are not predictive of outcome. A metastasis that is asymptomatic, solitary, less than 3 cm in diameter, metachronous, slow growing (long tumor doubling time), without lymphatic metastases, and a similar or lesser tumor grade than the primary RCC is more likely to be resected for cure. Absence of some factors should not preclude resection, however, the absence of multiple factors should alert a surgeon that resection is unlikely to be curative.

Biologic behavior of a RCC and the host's defense mechanisms cannot be evaluated during patient selection for pulmonary resection. These factors are major determinates of the behavior of a pulmonary metastases. Presently, they can only be approximated by the listed clinical predictors. Until an understanding of tumor and host behavior is achieved, patient selection will be an educated guess.

5. PREOPERATIVE PREPARATION

Prior to pulmonary metastasectomy, a careful history, physical examination, and routine blood work are completed. The status of local and metastatic RCC should be reevaluated by CT scans of the chest, abdomen, and pelvis, if scans are more than 8-wk old. A brain CT and bone scan should also be included because some nodules will be primary bronchogenic carcinomas and RCC that metastasize to brain and bone are usually lethal. Positron emission tomography (PET scanning) is still investigational, but, if available,

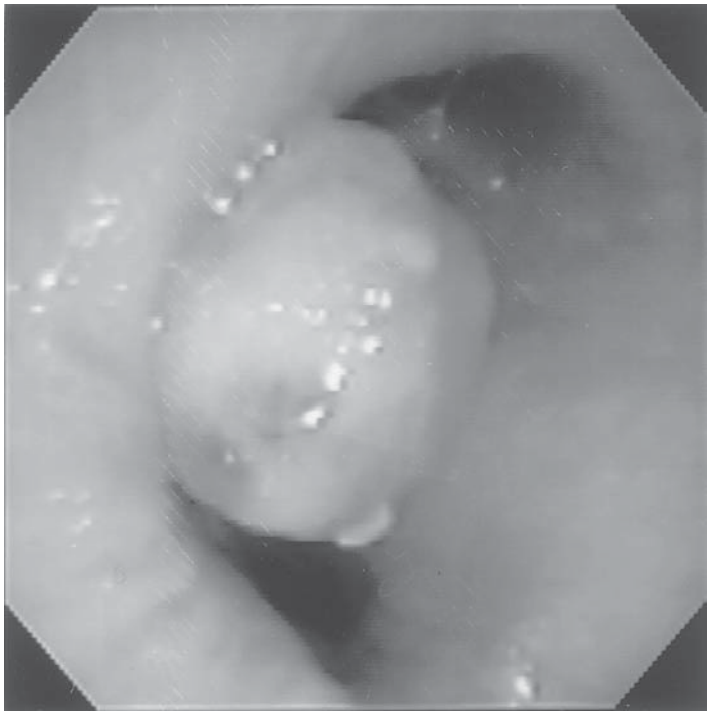

Fig. 4. Bronchoscopic evaluation of a patient who developed hemoptysis following nephrectomy. At the bifurcation of his left main stem bronchus, a endobronchial renal cell metastases can be seen.

it may be useful in the assessment of pulmonary nodules and exclusion of distant metastatic disease to other sites.

Spirometry and arterial blood gases are required for pulmonary assessment. In patients receiving preoperative chemotherapy, a diffusing capacity (DLCO) should be included in the pulmonary assessment. In patients with marginal lung function and in those requiring multiple or large resections, quantitative perfusion scanning allows an estimate of the pulmonary parenchyma that remains after resection. Exercise testing is recommended for patients with marginal pulmonary function. In patients over age 60 and in those with a cardiac history, a stress thallium study or dobutamine echo is advisable. Other studies will depend on comorbid conditions found during the history and physical examination.

Prior to resection, tissue diagnosis is not mandatory because a negative result for malignancy may reflect a false negative sampling error. If a diagnosis is required, the ascending order of sampling is sputum cytology, bronchoscopy with bronchial washings and brushings, transbronchial biopsy or percutaneous needle biopsy, and thoracoscopic biopsy. Sputum cytology is safe, easy, and well tolerated, but is more likely to provide a tissue diagnosis when the metastasis is central. Bronchoscopy should be performed prior to or at the time of resection to exclude endobronchial metastases or direct bronchial involvement *(25–27)* (Fig. 4). Although uncommon, endobronchial metastases are reported in up to 1.5% of patients with endobronchial malignancies and are twice as frequent as bronchial carcinoid *(28)*. Cytologic examination of washings and brushings may be diagnostic in peripheral lesions. Transbronchial biopsy or percutaneous fine needle aspiration may yield a cytologic diagnosis. Thoracoscopic excision of a pulmonary nodule is performed for diagnosis only and should not be used with curative intent *(29–31)*. The

diagnosis of a solitary metastases by CT scan and its excision at thoracoscopy will cause a 50% incidence of incomplete resections *(30)*. The inability to palpate the lung at thoracoscopy results in a 32% to 78% incidence of missed pulmonary nodules *(30)*.

Preparation for pulmonary resection includes smoking cessation and optimization of pulmonary function with bronchodilator therapy and cardiopulmonary rehabilitation.

6. OPERATIVE AND POSTOPERATIVE CARE

The surgical approach for unilateral metastases is a muscle-sparing posterior lateral thoracotomy. This provides excellent access to the ipsilateral lung and mediastinum. If lesions are bilateral, staged thoracotomies may be used. The least involved lung is approached first so that adequate pulmonary parenchyma is available for single lung ventilation when the major pulmonary resection is done approx 6 wk later. Bilateral metastases are generally approached via median sternotomy or a "clamshell " (bilateral anterior thoracotomies and transverse sternotomy) incision.

The possibilities of multiple metastases, undetected metastases, contralateral surgery or reoperation, and repeat resection mandate pulmonary sparing resections. Because the metastatic process is usually hematogenous, many metastases are peripheral and lymphatic spread is late. The standard resection is a nonanatomical wedge excision (Fig. 5). For deeper lesions, a segmentectomy or lobectomy may be required. A pneumonectomy is required for solitary hilar metastases, but carries an increased operative mortality and is unlikely to be curative in patients with bilateral pulmonary disease. Sampling or lymphadenectomy of hilar and mediastinal lymph nodes should be part of every pulmonary metastasectomy.

Pathologic evaluation of all resected nodules is essential for identification of benign and unrelated primary bronchogenic carcinomas. The typical histologic appearance of a clear cell renal carcinoma is generally reproduced in the metastases, however, it may be less well differentiated. Unlike bronchogenic carcinomas, RCC do not produce mucin. Therefore, a mucin stain may be helpful in determining the origin of an adenocarcinoma without clear-cell differentiation. With some adenocarcinomas, the diagnosis of a metastases following nephrectomy may only be by presumption. Sarcomatoid variants of RCC may be difficult to differentiate from primary or secondary spindle-cell tumors in the lung. Special staining may further define the process, but is unlikely to confirm the metastatic nature of the carcinoma.

Postoperative care of patients undergoing pulmonary resection of renal cell metastases is similar to that of all patients undergoing pulmonary resection. Hypotension and medications with known renal toxicity should be avoided.

7. RESULTS

Operative mortality ranges from 0% to 2.2% in patients undergoing resections of renal cell metastases *(22,24)*. Operative mortality increases with age greater than 70 yr and with pneumonectomy. Thirty-day postoperative mortality in the range of 1% can be expected for pulmonary metastasectomy. Operative morbidity has been reported in up to 9% of patients *(22)*. Following pulmonary resection 5-yr survival varies from 21% to 44% *(19,24)*. In carefully selected patients in whom all pulmonary metastatic disease can be resected, a 25% to 30% 5-yr survival can be expected. Resection of limited extrapulmonary disease does not adversely effect survival *(22)*.

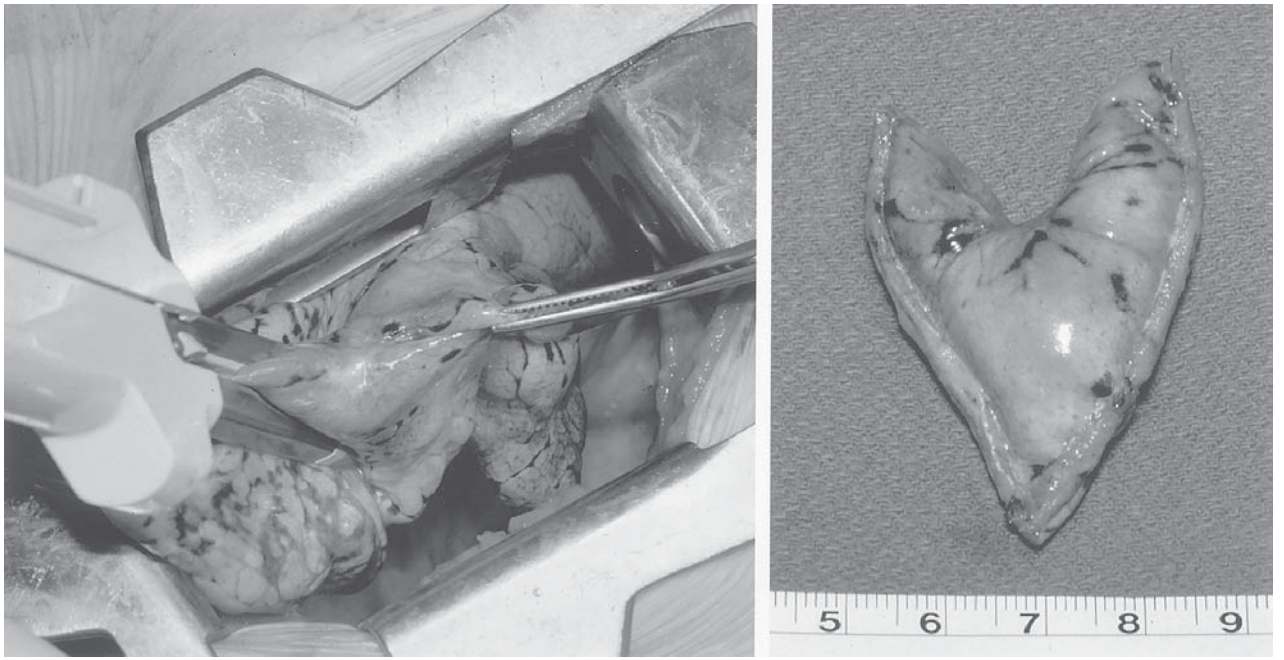

Fig. 5. At right thoracotomy, a pulmonary metastases is wedged using a linear cutting stapler. The resected specimen can be seen on the right. Pulmonary sparing resections are preferred in the treatment of metastatic carcinoma.

Table 1
Relative Risk of Death Following Pulmonary Metastasectomy

Tumor type	*Relative Risk of Death*	*95% Confidence Interval*
Teratoma	0.373	0.272, 0.510
Wilms'	0.503	0.232, 1.088
Embryonal	0.571	0.373, 0.829
Uterus	0.796	0.555, 1.142
Bowel	0.831	0.721, 0.959
Head and Neck	0.898	0.735, 1.096
Kidney	*0.928*	*0.790, 1.091*
Other Bone Sarcoma	0.965	0.789, 1.180
Osteosarcoma	0.990	0.863, 1.136
Synovial Sarcoma	1.026	0.833, 1.264
Leiomyosarcoma	1.098	0.878, 1.374
Breast	1.117	0.945, 1.320
Other Epithelial	1.120	0.900,1.393
Histiocytoma	1.150	0.937, 1.412
Other Soft Sarcoma	1.238	1.078, 1.422
Lung	1.374	0.913, 2.067
Melanoma	2.034	1.728, 2.394

Modified from: The International Registry of Lung Metastases, Pastorino U, Buyse M, Friedel G, et al. Long-term results of lung metastasectomy: prognostic analyses based on 5206 cases, *J. Thorac. Cardiovasc. Surg.,* **113** (1997) 37–49.

Unlike patients with metastatic osteogenic sarcoma, reresection of recurrent renal cell pulmonary metastasis is generally not indicated. However, in selected patients, repeat resection of pulmonary metastases has not reduced 5-yr survival *(22,24)*. The major cause of late death in most patients is recurrent carcinoma. This may be confined to the lung or primary site, but is usually disseminated. Compared to other tumor types, the adjusted relative risk of death of a patient with resected pulmonary metastases from RCC is intermediate (Table 1) *(16)*.

8. BIOLOGICAL THERAPY AND SURGERY

The spontaneous regression of metastatic RCC emphasizes the importance of immunologic factors in treatment of stage IV RCC *(32)*. However, cytoreductive surgery of the primary and metastases in preparation for systemic immunotherapy has not been effective *(33)*. Progression of disease and operative mortality and morbidity prevented 77% of patients from receiving planned systemic therapy. Following successful immunotherapy, surgical resection can be performed for metastases with the possibility of prolonged remission *(34,35)* (Fig. 6). Preoperative immunotherapy and pulmonary metastasectomy may not improve survival compared to metastasectomy alone *(22,35)*. Inhaled interlukin-2 and interferon have been effective in the management of pulmonary and mediastinal metastases, but their use prior to pulmonary metastasectomy have not been reported *(36,37)*.

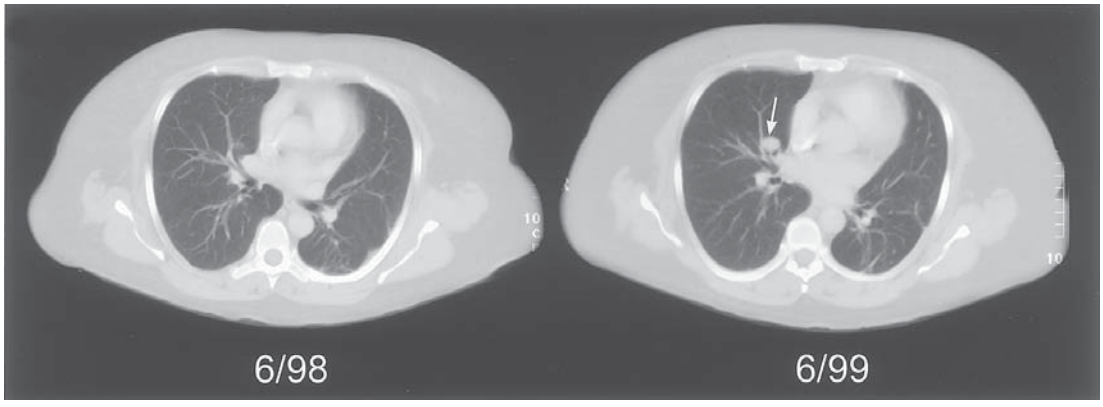

Fig. 6. These chest CT scans demonstrate the development of a pulmonary metastasis in the medial segment of the right middle lobe [arrow]. The patient has undergone nephrectomy, 5FU and IL-2 therapy and previous pulmonary metastasectomy. The right middle-lobe nodule was successfully treated with right-middle lobectomy. Incidentally, at operation "silent" metastases not seen on chest CT scan were resected from the right upper lobe.

9. CONCLUSIONS

Pulmonary metastasectomy is indicated in patients with stage IV RCC if the primary site is controlled, the lungs are the only site of metastases, all metastases are resectable, the patient's pulmonary status is adequate, and no major comorbidity is present. Preoperative immunotherapy may improve resectability and prolonged remission, but it is unlikely to improve survival. Determinates of survival are complete resection of all metastases, a single metastatic focus and a disease-free interval of more than 36 mo.

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16 Management of Skeletal Metastases in Renal Cell Carcinoma Patients

Michael J. Joyce

CONTENTS

1. INTRODUCTION

Patients with metastatic renal cell carcinoma (RCC) to the skeleton are evaluated by the orthopedic surgeon either directly because of unknown cause of skeletal pain or by referral from primary physicians, medical oncologists, or urologists after a lesion of bone is identified. Often a cancer diagnosis is well appreciated and the patient may have already had a nephrectomy and/or ongoing adjuvant therapy. Because of bone pain or a positive bone scan, the orthopedic surgeon is requested to evaluate and assess the structural integrity of long bones and the spine. In situations where a lytic lesion is visualized and the actual diagnosis is unknown, the surgeon embarks upon the differential diagnostic workup. Although all orthopedic surgeons are trained to diagnose and surgically manage metastatic disease, metastatic tumor cases can be just as challenging as primary sarcomas of bone and are often referred to orthopedic surgical oncologists, who are well versed in the complexity and management pitfalls of these lesions.

When a patient presents with a hole in the bone, a radiographic assessment is made on two views, anteroposterior and lateral views, of the involved bone with the entire bone

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being visualized. Typically, the lesion of RCC to the skeleton presents with a geographic lytic lesion and often times, with a significant soft tissue mass external to the bone. The radiographic appearance is that of a hole in bone with a permeative destructive margin without significant bony reactive sclerosis. The majority of renal cell metastases are indeed lytic allowing minimal bone healing response with little to no evidence of host reactive bone to the destructive tumor component. The renal carcinoma cells themselves do not actually destroy bone, but produce humoral proteins that can recruit and stimulate host osteoclasts to destroy bone locally. Carcinoma cells permeate between the trabecular bone as an infiltrative and destructive process. However, the osteoclasts of the host and the resident macrophage precursors (tumor infiltrating macrophages *[1]* induced into osteoclastogenesis by the tumor cells) are the probable cause of the bone destruction *(2)*.

Even though major orthopedic tumor centers see a high frequency of primary bone tumors such as osteosarcomas, malignant fibrous histiocytomas, and chondrosarcomas in adults, lytic destructive lesions in the skeleton are usually metastatic lesions from a solid organ tumor. When one compares the incidence of metastatic skeletal lesions from solid organ tumors to that of primary malignant bone lesions in patients over age 40, the ratio of this being a metastatic lesion rather than a primary bone tumor is well over 100:1. Bone scans depicting multiple areas of involvement in the skeleton further increase the likelihood of the lesion being metastatic disease. However, it is not unusual for a RCC to initially present as a solitary lesion to the skeleton. Most of these lesions are geographical metaphyseal destructive lesions eccentrically placed. However, isolated cortical metastases are not uncommon for renal cell. Lung carcinoma is the most common intracortical metastases with renal cell occasionally causing this appearance. Some of the renal cell metastatic lesions do not present as lytic lesions, but as a permeative type appearance with diffuse loss of cortical and trabecular bone substance. Skeletal metastatic lytic lesions below the knee and elbow are more likely to be from the lung, but RCC metastases are not that rare distal to these areas.

Concerning the patient with multiple bone lesions, one should be cautious to make sure the patient does not have severe osteopenia with loss of trabecular bone, thinning of the cortices, and multiple lytic destructive cystic lesions. Most likely in these particular cases, the diagnosis is that of metabolic bone disease of hyperparathyroidism with an elevated parathyroid hormone and a multifocal positive bone scan. The bone left between metastatic lytic areas should indeed be normal and not have the plain film radiographic appearance of metabolic bone disease.

On review of frequency of bony metastatic disease, breast carcinoma is the most common lesion for the orthopedic surgeon to manage in females, with prostate being most common in males. Metastatic breast lesions can be either blastic presenting as radiodense "whitish" lesions, purely lytic, or mixed. Ninety percent of skeletal prostate lesions are blastic. Lung metastatic disease is the second most common for the sexes. These lung lesions present as radiographic destructive lytic lesions, although occasionally some of the lung lesions may indeed be blastic. Renal cell metastases are a very distant third in the sexes concerning occurrence of metastatic disease that is brought to the attention of the orthopedic surgeon.

2. INITIAL EVALUATION

Patients who present with a lytic destructive lesion undergo a typical history and physical examination. This would include a smoking history; questions directed to change

in bowel habits and blood in the stools; cough and sputum production; thyroid problems and neck masses; history of urinary voiding problems/prostatitis if a male; and family breast history of cancer, known lumps or nipple discharge/retraction if a female; and especially for RCC, flank pain, and gross hematuria. In the examination looking for the source of a metastatic bone lesion, astute attention should be directed toward ruling out lung carcinoma, especially with a chest X-ray; breast problems in females looking for nipple retraction and discharge, breast masses, and axillary adenopathy; voiding problems, enlarged nodular prostate gland in males; palpating for thyroid/neck masses; and palpating abdominal masses, and rectal examination with stool guaiacs. The physical examination for RCC of the kidney usually does not yield a palpable mass nor flank pain on percussion, but microscopic hematuria may indeed be appreciated on urinalysis.

Laboratory studies in the way of acid phosphatase and prostatic specific antigen can be helpful for the skeletal metastatic prostate lesion especially in the 10% lytic cases. Most myeloma lesions have a distinct radiographic appearance, but serum protein electrophoresis, urinary protein electrophoresis, and immunofixation studies can be helpful in addition to looking for significant anemia on the complete blood count for the suspicion of myeloma. Often the laboratory studies show an elevated total protein and a lower albumin in the myeloma patient. The radiographic appearance of a large destructive lesion without margination in a renal cell metastasis is usually quite different than that of the punched out smooth border nonsclerotic margin of a myeloma lesion. However, it may be quite difficult to discriminate radiographically between the permeative lesion of bone for renal cell compared to a permeative lesion of myeloma. CT scans of the lung, abdomen, and pelvis can be helpful in identifying the solid organ primary lesion that has caused the skeletal metastatic deposit.

3. ROLE OF THE BONE SCAN/RADIOGRAPHIC IMAGING

At the present time, total body bone scans are not thought to be cost effective in the routine staging investigation for patients with renal carcinoma unless skeletal bone pain symptoms are present. Studies have demonstrated that the vast majority of true positive lesions are associated with bony discomfort. Alkaline phosphatase is an insensitive indicator of bone metastases as demonstrated by Kriteman and Sanders *(3)* . Bone metastases were demonstrated in 164 of a cohort of 539 renal carcinoma patients. Alkaline phosphatase levels were less than or equal to 141 U/L in 72% and less than or equal to 111 U/L in 53%. In the second cohort of 184 patients, 22 of 37 bone metastases patients (59%) had little to no bone pain at presentation and 86% had a normal alkaline phosphatase. This incidence of occult skeletal metastasis without bone pain is in sharp contrast to Henriksson's *(4)* review of 102 patients with renal carcinoma of whom 33 patients (32.4%) had metastatic spread, but bone metastases were found in only six patients, 5.9%. All six patients were symptomatic with bone pain and routine bone scanning in 70 patients demonstrated no other sites of skeletal metastasis. Seaman et al. *(5)* conducted a retrospective review of 28 of 90 patients with a positive bone scan who were under therapeutic treatment. Thirty-nine percent had a normal alkaline phosphatase and three of these 11 patients had no bone pain. Of these three asymptomatic patients with bone metastases and normal alkaline phosphatase levels, only one had bone as the only site of renal metastasis and would have been incorrectly staged without a bone scan. The conclusion was that a bone scan may be safely omitted in patients with renal carcinoma with normal alkaline phosphatase levels and no bone pain. Sandock et al. *(6)* reviewed 158

patients retrospectively who had a radical nephrectomy with 137 having no evidence of metastasis at diagnosis. Even though disease recurred in 52.8% of their $T_3M_oN_o$ group, the article suggested that the routine use of bone scans and computerized tomographs did not appear necessary for follow-up and decisions for imaging could be made on an individual clinical basis.

Newer serum markers for bone loss, such as pyridinoline and deoxypyridinoline crosslinks may be used as a discriminatory factor in the decision making process for requesting a total body bone scan. In contrast to renal carcinoma not inciting a limited bone reaction process leading to an elevated alkaline phosphatase, Nemoto et al. *(7)* have demonstrated significant correlation between the bone loss radiographically and the level of pyridinoline crosslinks in the animal model.

Because of the low sensitivity of the technetium bone scan for very early skeletal metastasis without radiographic abnormality or bone pain, magnetic resonance imaging (MRI) has been used as a tool to discriminate sites of potential bony metastasis. Other radiopharmaceuticals such as Ga-67 and Yttrium-90 seem to have better affinity for renal cell metastasis both for imaging and possible therapeutic treatment *(8)*.

The most sensitive tool for imaging of occult metastatic disease in the radiology armamentarium is MRI looking for spin signal changes of infiltration. An MRI can be helpful in discriminating between stress fractures and solid organ metastatic disease. The MRI is quite helpful in defining the extent of the soft tissue mass, epidural spinal masses, and other infiltrative bone lesions, especially in evaluating extent of disease in other vertebral bodies of the spine. Spine lesions most often present as lesions of the anterior column with involvement of the vertebral body with eventual pedicle destruction. Identifying and treating epidural spine compression lesions prior to the patient having a neurologic deficit is important in reducing the morbidity of these epidural lesions.

A simple fine-needle aspiration either under fluoroscopy or CT scan can be very helpful to the management team in defining the lytic lesion as being metastatic disease. Accuracy is well above 95% when identifying these lesions as metastatic. Immunoperoxidase staining can be used in addition to the cellular morphology in differentiating between renal cell and other solid organ metastatic disease.

4. CLINICAL PRESENTATION

Presentation of some of these lesions can be quite difficult to discern for the clinician, the radiologist, and the orthopedic surgeon. This is especially true in the younger-age patient. Bone lesions about the pelvis can be confused with sciatica and leg pain. Because of the extensive destructive nature of the renal cell metastases, the metastatic lesion is associated often with a large soft-tissue mass. Illustrated (Fig. 1) is a 39-yr-old gentleman who underwent a laminectomy at L5/S1 for a bulging disk for his left leg pain five months prior to being evaluated for continued back and pelvic left buttock pain. Plain film radiograph showed a lytic lesion of the iliac wing adjacent to the posterior iliac crest. The total body bone scan showed only a single isolated lesion. The bone scan demonstrated a “cold” central area of limited uptake of the radionucleotide surrounded by a rim of increased uptake. The CT scan showed some periosteal reactive bone rimming the large expansile mass making an aneurysmal bone cyst a reasonable differential diagnosis. Because the MRI demonstrated the expansile lesion to be predominantly fluid filled, the working diagnosis because of the faint periosteal shell around a fluid-filled mass was that

of an aneurysmal bone cyst. A fine-needle aspiration yielded only blood with minimal cells and a presumptive diagnosis of aneurysmal bone cyst. On the open biopsy, blood and clot were predominantly found. A frozen section of the lining demonstrated RCC. The surgeon was prepared for prompt curettage. After curettage of the bulk of the lesion with removal of the periphery of the renal cell metastasis, bleeding usually ceases as occurred in this case.

Orthopedic surgeons should be cognizant of the potential diagnosis of a pathological fracture during routine fracture management. Illustrated (Fig. 2) is a 42-yr-old gentleman who fell on the ice sustaining an intertrochanteric hip fracture. In retrospect, the fracture films suggest a preexisting lytic lesion of the proximal femoral area. The fracture was stabilized with a sliding nail side-plate device. Only in retrospect a week later after a call from the radiologist, did the orthopedic surgeon appreciate that the postoperative films indeed suggested a cavity. Apparently, no excessive bleeding occurred during the fixation to alarm the surgeon. The search for a solid organ tumor was embarked upon with CT scans of the chest and abdomen with a large mass in the kidney being identified as RCC. The patient subsequently underwent a nephrectomy. Because of the bone loss, the patient was treated in traction for 6 wk and was subsequently in a spica cast for an additional 6 wk with the oncologist stating that the patient would not survive long enough to warrant definitive management of his proximal femur after hardware failure. The patient was referred 3 mo later with a large soft-tissue mass about his left hip. Embolization was accomplished and the large mass resected including his proximal one-third femur similar to what would be accomplished for a primary malignant bone tumor procedure. The limb was reconstructed with a proximal femoral replacement component. The patient was quite functional for 2˚ yr before subsequently succumbing to systemic metastatic disease with the construct just lasting for the duration of his life. This illustrates the importance of sending tissue intraoperatively for frozen section and eventual permanent section in order to diagnose the possible cause of the fracture if there is any suspicion of a pathologic fracture.

The predominant musculoskeletal problem pertaining to renal cell metastases relates to loss of function and pain. Pain can be related to the expanding lesion within the medullary canal with the huge soft tissue mass causing pressure on the pain fibers within the periosteum. This is a pressure induced phenomenon related to the pushing borders of the tumor. For the somewhat limited radiosensitive lesion of renal cell metastasis, radiotherapy can still be somewhat effective in causing a shrinkage of the tumor, thus temporarily relieving pain related to the expansile pressure on the periosteum. The second source of pain is that of mechanical structural pain related to an impending fracture of the bone. Whereas radiation therapy may relieve the mass effect of the tumor upon the periosteum, the mechanical and structural pain related to bending and compression of the bone will not be relieved with radiation therapy and is manifested by increased pain with attempts at weight bearing. For tumors that are fairly radiosensitive, the pressure-type pain is promptly resolved with the course of radiation therapy to about 30 Gy. The radiation therapy hinders bone healing for a number of weeks, but unless the pushing destructive borders of the tumor are eliminated, there would be no bone healing. In RCC, the tumor is only partially radiosensitive. One of the goals would therefore be to prevent a large amount of bone destruction before there is excessive loss of structural integrity of the bone. Efforts should be coordinated as a team approach (Fig. 3) with the radiation oncologist, medical oncologist, and the orthopedic surgeon for assessment of early bone lesions before they become large difficult lesions for the orthopedic surgeon to manage.

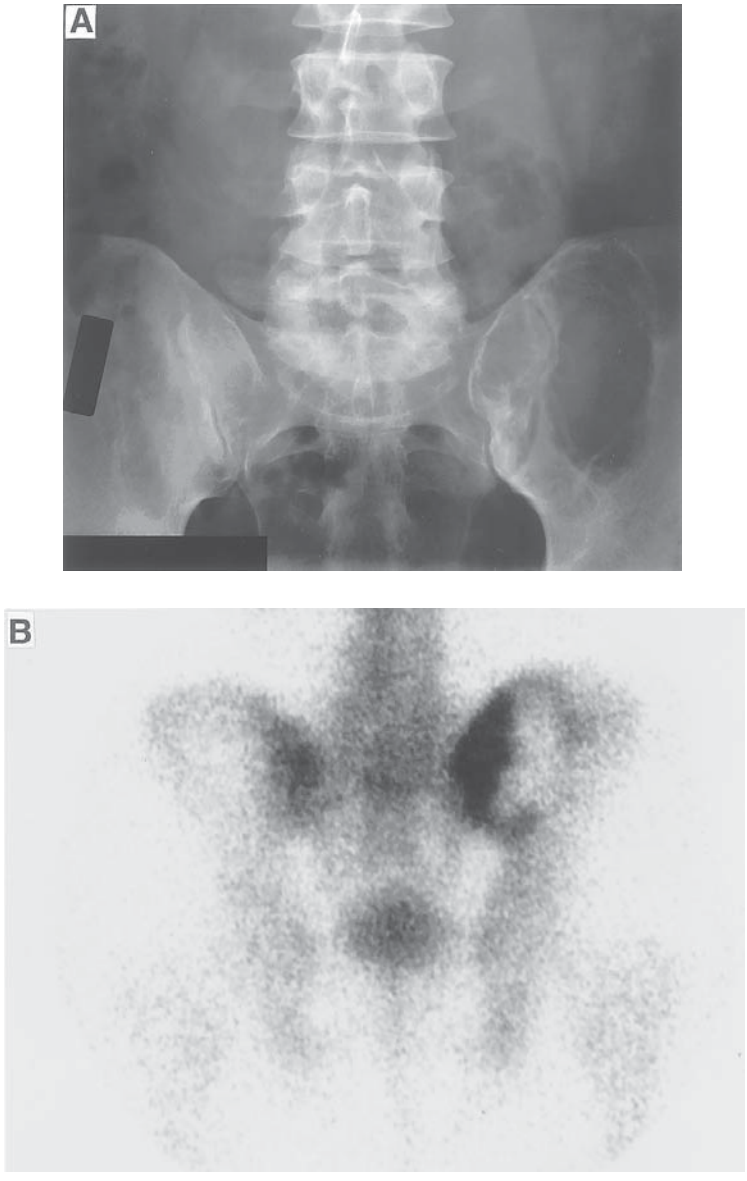


Fig. 1. (**A**) Radiograph of a lytic expansile lesion of the posterior left pelvis in a 39-yr-old male. (**B**) Bone scan demonstrates a photopenic central area with surrounding increased uptake of radionucleotide.

5. SURGICAL STABILIZATION AND MANAGEMENT

The treatment axiom for stabilization of lesions of the appendicular skeleton is that of rigid fixation. The ideal location for hardware fixation devices should be intramedullary where the neutral bending axis force exist. In light of loss of significant bone substance, methylmethacrylate (bone cement) is commonly used to provide immediate stable fixation. RCC metastatic to bone are only partially radiosensitive and one should not plan on future bone healing to resolve situations of marginal stable internal fixation. The goal is

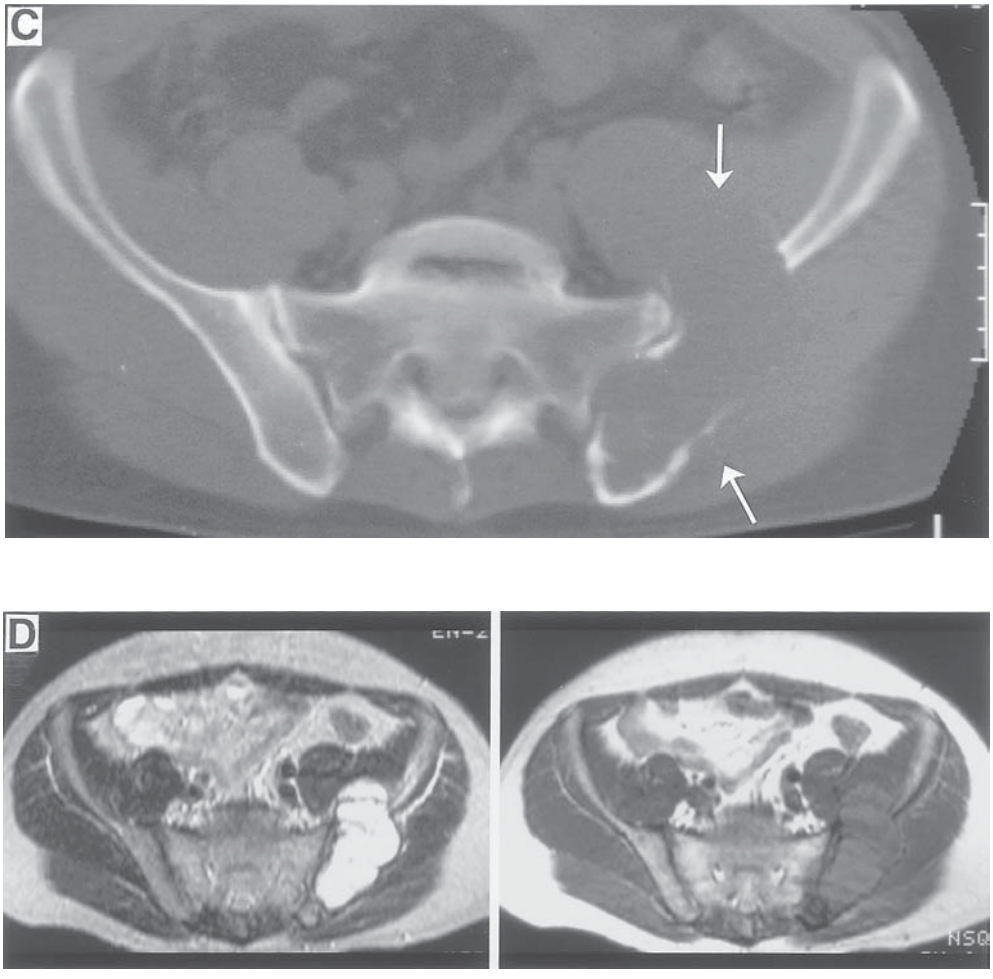


Fig. 1. (Continued). (**C**) CT demonstrates a large expansile soft-tissue mass with some peripheral periosteal bony shell around the lesion. (**D**) MRI shows a fluid filled lesion with a peripheral rind around the area simulating a possible aneurysmal bone cyst.

to make the construct as stable as what it will ever be at the time of the surgical case in light that one cannot count on a bone-healing response with renal cell metastases. The construct of plates and screws even with methylmethacrylate bone cement to stabilize the metastatic lesion is doomed to failure once there is further bone destruction. Illustrated (Figs. 4–6) are failures of fixation devices used for fracture work with progressive bone destruction leading to loss of fixation, deformity, and further pain within weeks and months of the original stabilization procedure.

In light that fixation devices depend upon the integrity of the bone structure, the appropriateness of internal fixation should be reserved for those lesions that remain somewhat structurally intact. Often times, the bony outer shell of a lytic lesion can function as a container for methylmethacrylate if intramedullary rodding is planned such the that length of a bone is preserved. Planning for prophylactic fixation allows the surgical case to be done on an elective basis with the patient optimized concerning medical conditions. If one waits for an acute fracture before proceeding, often there is significant bleeding,

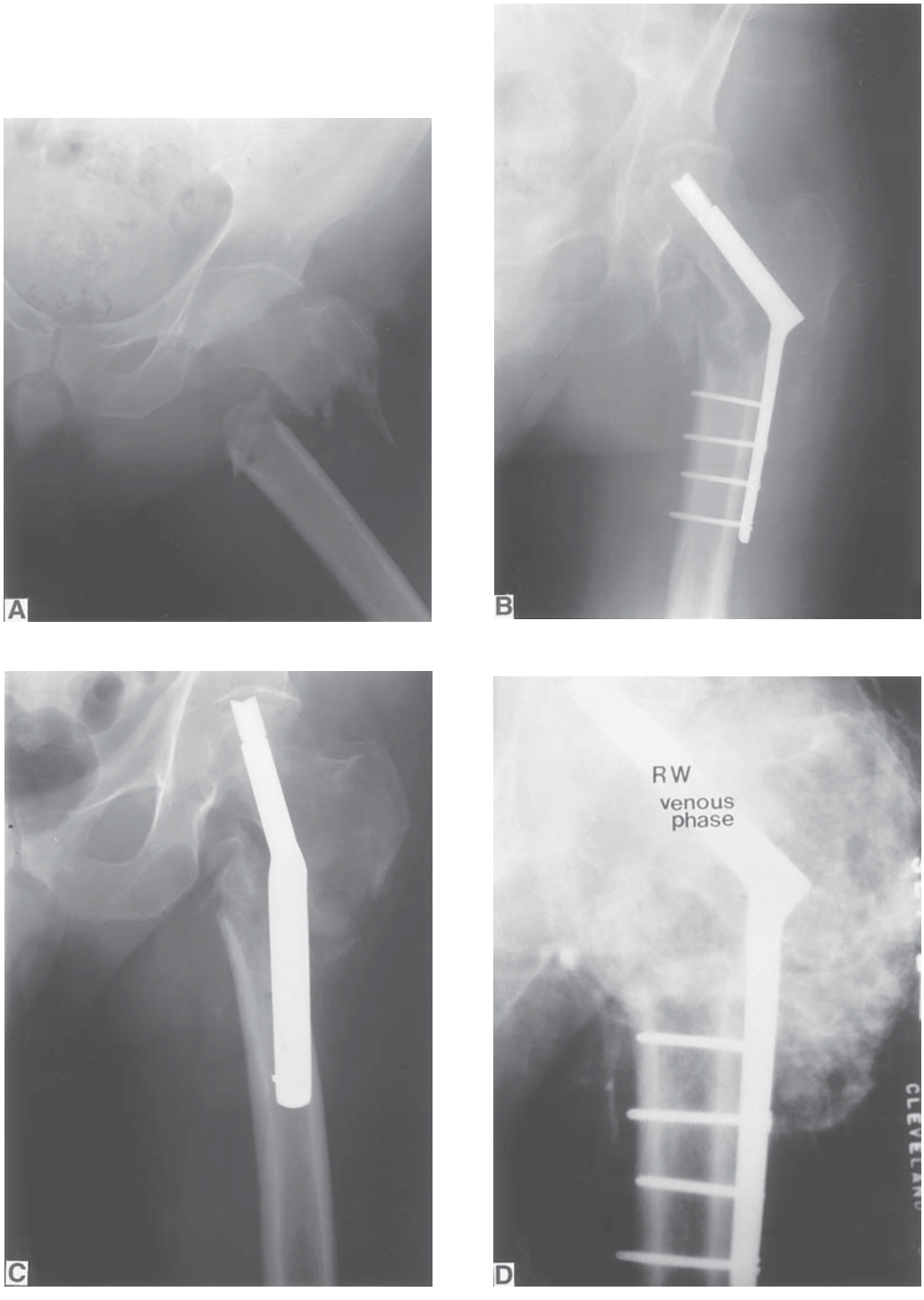


Fig. 2. (**A**) 42-yr-old male sustained an atypical intertrochanteric hip fracture. (**B**) Internal fixation performed with a nail/plate sliding device. Areas of bone loss are evident. (**C**) Fracture fixation failure at 6 wk. (**D**) Venous phase arteriogram showing huge soft-tissue mass just prior to embolization.

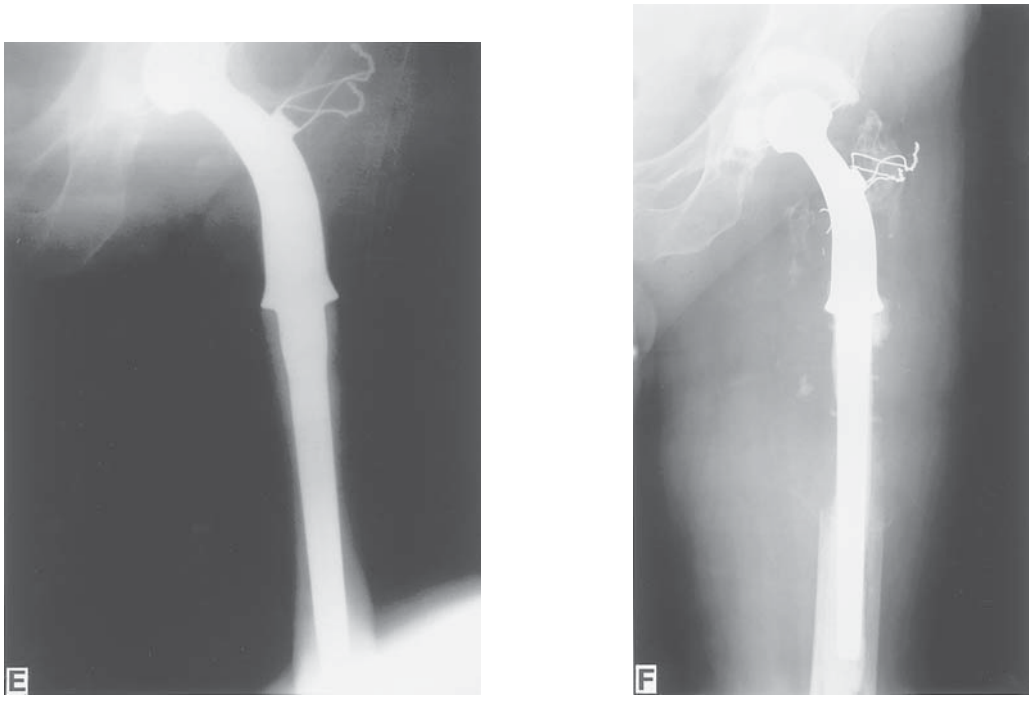


Fig. 2. (Continued). (**E**) Early postoperative film with restoration of femur by a proximal femoral replacement with a total hip acetabular cup because the nail destroyed acetabular articular cartilage. (**F**) Radiograph 30 mo later with patient fully ambulatory with use of a cane. He died three years after en bloc resection and reconstruction. Bone destruction around the cemented stem is evident, but the component remained fixed to bone.

pain, and suffering such that the orthopedic surgeon is directed to fix things immediately for pain relief for the patient. If bones are stabilized with intramedullary rods and cement before fracture, length and integrity of the bone can be preserved, and often the operative intervention is much less stressful both on the patient, as well as the surgeon. Early use of radiation therapy to prevent massive bone loss before there are huge destructive lesions can be helpful. The medical oncologist, radiation therapist, and the orthopedic surgeon well versed at metastatic disease function as a team. Decisions should be made early to prevent bone loss and decide who could functionally benefit from early surgical intervention of their metastatic skeletal lesions.

Early recognition of impending pressure on the spinal cord is imperative. Screening MRI scans can be quite helpful in defining the risk of skeletal spinal involvement. Although the MRI is quite sensitive in defining metastatic disease in bone and epidural masses, plain films and CT define the overall structural integrity and stability. Therefore, plain films, MRI scans, and CT scans are quite helpful to the spine surgeon as he or she plans for decompression and stabilization. In the cervical spine, surgical intervention from an anterior approach is accomplished using methylmethacrylate, autograft, allograft, or cage to create stability. The anterior column is involved in metastatic disease and the anterior column needs to be restored for structural integrity. The surgical decompression and stabilization should be done by spine surgeons well versed in spinal stabilization. Often, more than one level needs to be decompressed. The neck can be stabilized with

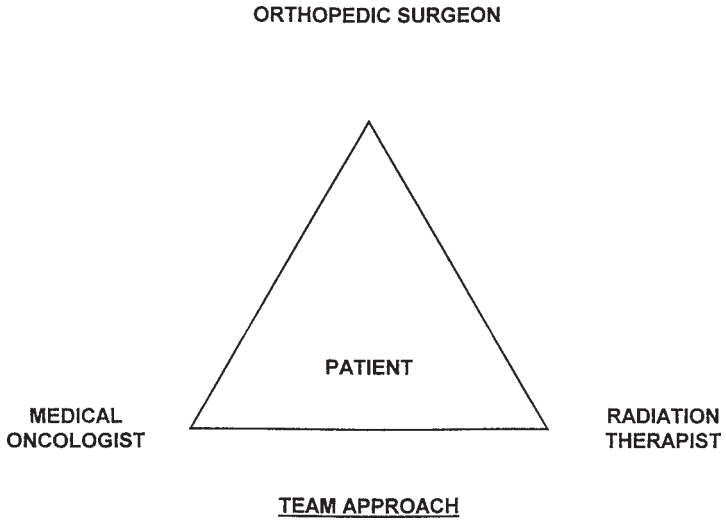


Fig. 3. Management of skeletal metastases involves a team approach of the medical oncologist, radiation therapist, and orthopedic surgeon.

large strut grafts stabilized by a spinal plate as supplementation. Concerning the thoracic spine, anterior approaches are a rule for decompression and stabilization. Frequently, the patient subsequently has a posterior pedicle screw or a laminar wire stabilization such that both the anterior and posterior columns are protected. Lumbar spine involvement can be approached either anterior or posterior. It is inappropriate to just do simple decompression of the posterior elements. This will destabilize the spine and put the patient at further risk for cord or nerve root problems. Many times anterior column stabilization is done through a transpedicle approach with posterior pedicle screw stabilization dependent upon the quality of bone. The surgeon may plan for both an anterior decompression with stabilization and a posterior stabilization at different settings to stabilize the spine. Patients are much more likely to recover neurologically from surgical management of epidural metastatic disease before complete progressive neurological loss in contrast to trying to retrieve a patient who has become paraplegic and has already lost bowel and bladder function. Therefore, close surveillance and early intervention is the rule in order to preserve motor and sensory function of the extremities in addition to bowel and bladder function.

6. EMBOLIZATION

Metastatic RCC causes an angiogenesis response yielding a very hypervascular tumor, often leading to significant intraoperative bleeding. Embolization can be quite helpful in renal cell metastases to reduce blood loss for both extremity and spine lesions. A number of articles *(9–11)* have shown that blood loss can be reduced 50 to 70 percent with the radiologist astutely embolizing feeder vessels to the metastatic lesion. Care needs to be taken concerning avoiding excessive embolization rendering an infarction to the spinal cord. Clinically, there does not seem to be a significant problem with regard to the concern of potential spinal cord infarction. The procedure of preoperative embolization is usually done near the time of the planned surgical intervention either the morning of or the day before the planned surgical case. New collateral circulation can be obtained

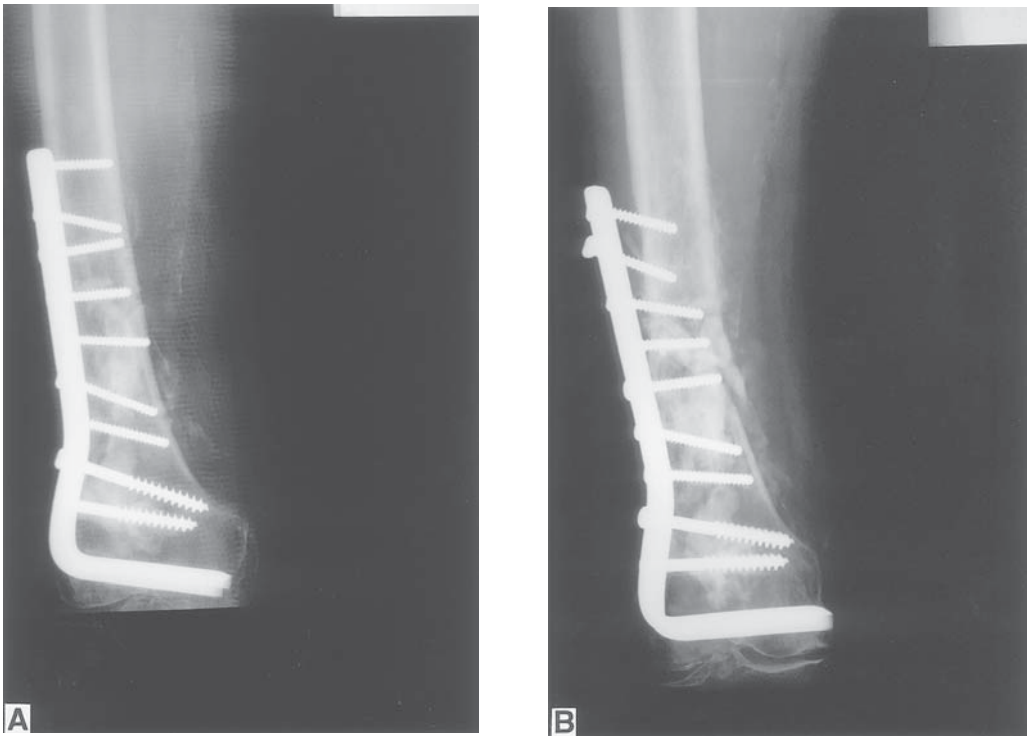


Fig. 4. (**A**) A 60-yr-old male with attempted internal fixation as a fracture supplemented by methylmethacrylate. (**B**) Failure of fixation within 3 mo.

by the tumor within a fairly short period of time. Embolization is usually done with nonabsorbable materials. Illustrated (Fig. 7) is the lumbar spine of a 70-yr-old man who underwent two courses of embolization, and both anterior/posterior column spine stabilization with a limited amount of blood loss intraoperatively.

7. FIXATION AS FRACTURE VERSUS REPLACEMENT

Metastatic RCC lesions are often near the end of the bone adjacent to the joint. This makes fixation with fracture techniques and rodding quite difficult. Lesions about the hip that involve the lesser trochanter and greater trochanter and femoral neck should be replaced and not treated as fractures. Often, a significant portion of the metaphyseal bone is involved and modular systems that supplement total joint systems are used. In this way, the components can replace significant bone loss areas with muscle attachments being reapproximated and the patient then allowed early weight bearing rather than be protected through a "fracture-healing process." Fracture healing may never happen. No matter what device is planned for use, the surgeon needs to be cognizant of the next area of bony failure whether it is at the tip of the rod or the tip of the device. The entire bone is frequently protected by an intramedullary stem device. This includes the femoral neck when rodding of the femur is performed (Fig. 8). When the lytic lesion is more in the shaft, rods and cement are used as illustrated (Figs. 9 and 10).

Fig. 11 is an example of distal femur with a large soft-tissue mass evident by the plain film and the resected distal femur. A modular distal femoral rotating hinge total knee

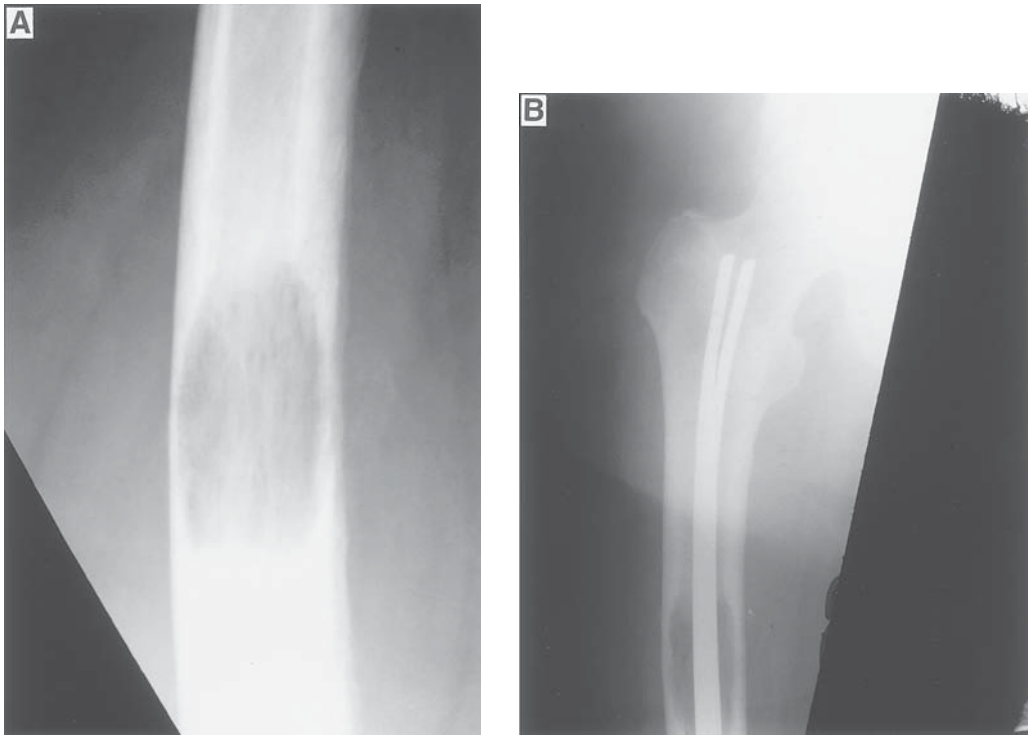


Fig. 5. (**A**) Painful midshaft lytic femoral lesion in a 62-yr-old male. (**B**) Enders nails with inadequate stabilization. Patient eventually fractured completely and was "too sick" to be operated upon. His final 2 mo alive were at bed rest, in pain.

system was used to reconstruct this distal femur allowing the patient early full weight bearing. This construct lasted the patient until he died of systemic disease 12 mo later. Fig. 12 illustrates the rapid progression of a renal cell metastasis that was ignored. The patient was functional at 14 mo later with a proximal femoral replacement after resection, but had systemic tumor involvement.

There is controversy concerning adjuvant postoperative treatment. It is well appreciated that large bulky RCC lesions are not all that radiosensitive. Radiation therapy usually to about 3000 cGy is the norm with the field irradiated to include areas of the bone in which tumor may have been pushed further down.

8. SOLITARY SKELETAL METASTASES

The presentation of a patient with RCC with a solitary metastasis can be a dilemma for the treating team. From review of the literature and anecdotal experience, a true isolated solitary metastasis is more likely to present remote in time from the definitive management of the primary renal cell tumor. It is common for other skeletal lesions to eventually be identified as time passes. The bone scan is not a very sensitive tool in identifying occult skeletal metastases that have normal bone radiographs. Some of these lesions are truly semidormant within the intramedullary or metaphyseal bone and may possibly only be visualized with MRI studies.

The approach of proceeding with an intralesional curettage procedure or a resection en bloc procedure for potential cure is a consideration for the orthopedic surgeon. Assistance

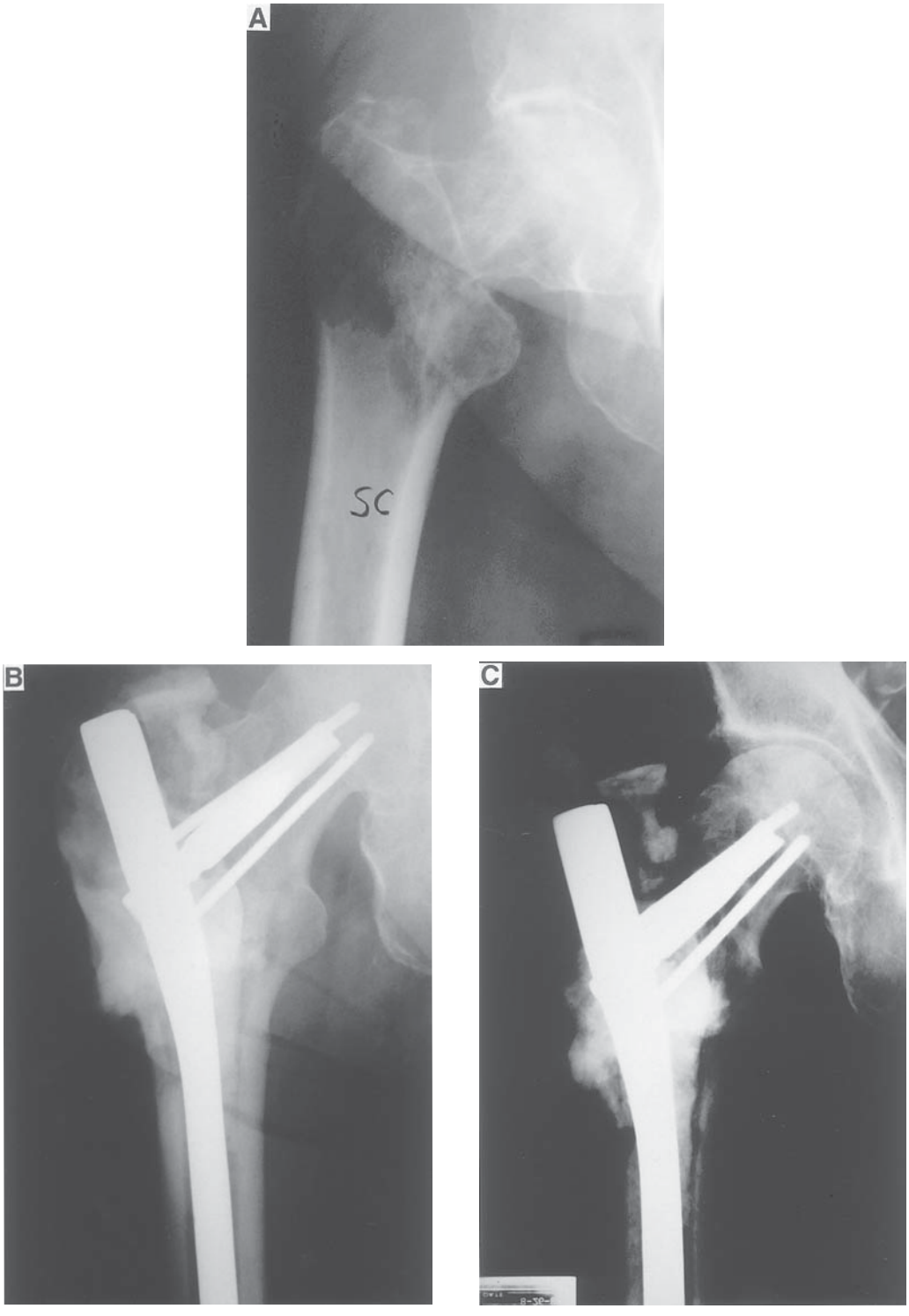


Fig. 6. (**A**) Involvement of the proximal femur in a 62-yr-old female. (**B**) This was internally fixed with a Zickel nail/rod device supplemented with stout rods up the femoral neck anchored with methylmethacrylate for fracture fixation. Patient received radiation therapy postoperatively at 4 wk. (**C**) Failure because of bone loss with severe pain 5 mo later. Only the methylmethacrylate and hardware remain in the intertrochanteric area.

should be provided by the team in coming to a mutual understanding whether this identified lesion is truly an isolated metastatic deposit. Once the patient undergoes clinical staging and is declared otherwise disease-free regarding bone scans, CT scans of the chest, abdomen, and pelvis, and possible MRI, it is appropriate to consider a tumor en bloc resection for a cure. This implies that the tumor will not be spilled in the field and a cuff of normal tissue will be obtained around the solitary metastatic deposition similar to that of an en bloc tumor resection for a primary bone sarcoma. This may require sacrifice of surrounding normal structures. This is in distinct contrast to an intralesional curettage procedure and filling holes with methylmethacrylate anticipating a much shorter longevity for the patient. For these en bloc "curative" procedures of the limb or pelvis, allografts or metallic modular components are used for reconstruction to improve function. Fig. 13 illustrates an en bloc resection of the left pelvic for cure and reconstruction with a pelvic allograft/total hip replacement. There are no large series of isolated renal cell metastases treated for a cure and most are incidental long-term outcomes from series of orthopedic management cases. More often than not, the treating team is disappointed to see new skeletal lesions appear within 2 yr; clearly signifying that the original skeletal lesion was not a true isolated metastasis.

9. SPONTANEOUS REGRESSION OF SKELETAL METASTASES AFTER PRIMARY MANAGEMENT OF RENAL HYPERNEPHROMA

Previous anecdotal reports of spontaneous regression of metastatic bone lesions after nephrectomy have been published. The biology of this phenomenon remains unclear. However, the reports clearly suggest that a local process occurs at the metastatic site in which the bone destruction is actually reversed.

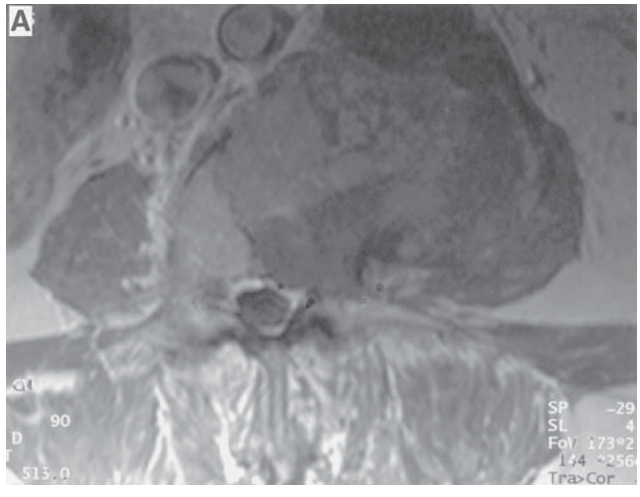
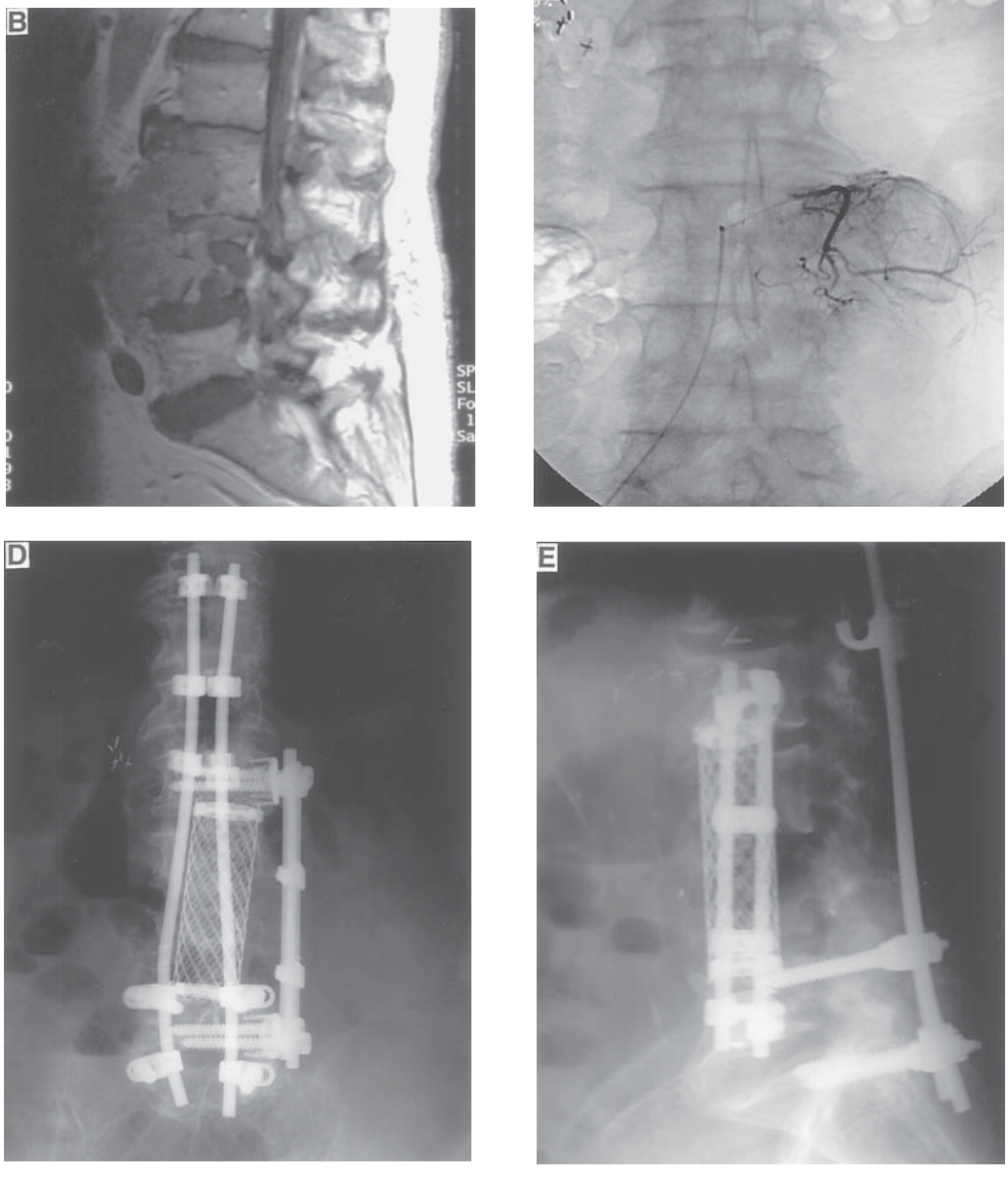


Fig. 7. (**A**) and (**B**) Axial and lateral magnetic resonance views of the lumbar spine in a 70-yr-old man with metastatic renal cell axial carcinoma. The lesion involved two adjacent vertebral bodies, but the patient otherwise had no evidence of systemic disease. The patient presented with progressive pain and inability to ambulate, but did not have a frank cauda equina syndrome.



(**C**) Angiography of the renal cell lesion demonstrating tumor blush and the neovasculature associated with the metastatic lesion. Two courses of embolization prior to surgeryrendered the lesion almost ischemic at the time of excision and the tumor mass was excised with less than 500 cc blood loss. (**D**) and (**E**) AP and lateral views status post tumor excision and reconstruction. Surgeons performed a two-level en bloc vertebrectomy, followed by anterior vertebral reconstruction with a titanium mesh cage and anterior screw and rod construct. The patient then had posterior stabilization with segmental instrumentation. At 6 mo follow-up, the patient was ambulatory, pain-free, and had completed a successful course of adjuvant radiotherapy. (Case from Dr. Robert McClain, Cleveland Clinic Orthopaedic Spine Surgeon.)

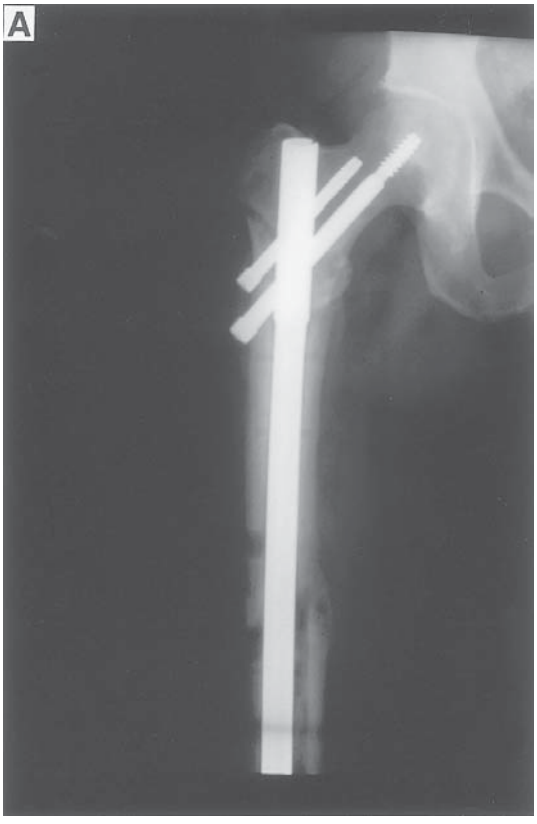


Fig. 8. (**A**) Protection of the entire bone includes the femoral neck. This is a reconstruction femoral rod with proximal and distal interlocking.

10. SURVIVAL AFTER DIAGNOSIS OF METASTATIC DISEASE OF THE SKELETON

Not only can orthopedic surgical intervention of the skeleton provide improvement in function and increasing the time before significant disability, procedures can also provide pain relief.

An earlier illustration was given concerning the misperception of survivorship of patients with renal cell metastases of the skeleton. With appropriate treatment, a number of these patients will do well for an extended period of time. Skinner et al. *(12)* reported an 8% 5-yr and 7% 10-yr survival rate in a 1971 published series. Thompson et al. *(13)* in 1975 reported survival rates of 21.5% at 1 yr and 9% at 2 yr, and 0% at 5 yr in a cohort of 65 patients. Dekernion et al. *(14)* in 1978 reported survival rates of 42% at 1 yr and 13% at 5 yr. Maldazys and Dekernion *(15)* in 1986 reported a 48% 1-yr survival and 9% 5-yr survival in a series of 181 cases. Tobisu et al. *(16)* in 1989 presented a group of patients with bony metastases, of whom 77% were alive 1 yr after presentation for bone involvement, 45% at 5 yr, and 0% at 10 yr. Smith et al. *(17)* in 1992 reviewed 14 patients with hypernephromas and skeletal metastases. Survivorship after skeletal metastasis at 1 yr was 58% with a range from 7 to 64 mo.

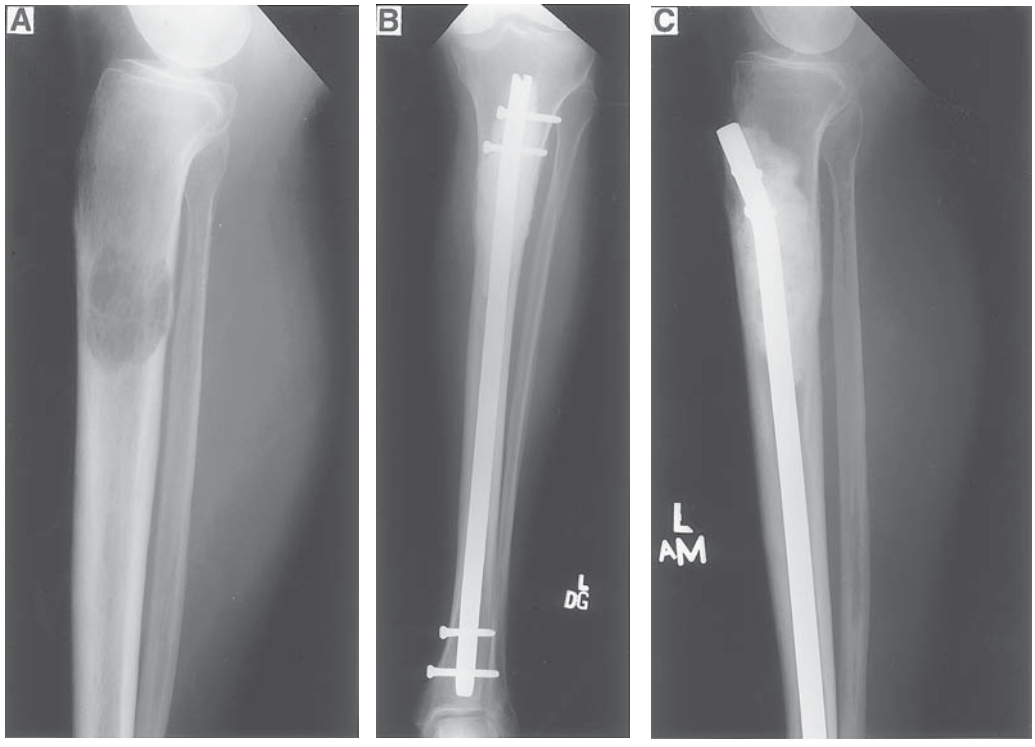


Fig. 9. (**A**) Lytic lesion in a 59-yr-old male proximal tibial shaft. (**B**) and (**C**) Asymptomatic patient with radiographs at 9 mo postoperatively. Patient received 3000 cgy 4 wk postoperatively.

The review of management of 38 renal cell patients with metastases to bone from the Massachusetts General Hospital (MGH) provides insight to a significant change in philosophy concerning management of these patients. Many urologists and orthopedists in the past consider RCC patients with metastasis to bone having a terrible prognosis and management of bone lesions should only be palliative. However, survival for the entire group aggressively managed surgically at MGH was 90% at 6 mo, 84% at 12 mo, 55% at 5 yr, and 39% at 10 yr. Original presentation without initial bony metastases, long disease-free period between nephrectomy and first metastases, appendicular rather than axial skeletal location, and solitary presenting metastases were correlated with better long-term survival.

When metastatic lesions are large and encompass a significant amount of structural bone loss, operative procedures directed at resection of the metastatic deposit with sacrifice of the bone segment often yield better long-term results when compared to intralesional procedures involving internal fixation with methylmethacrylate. Figs. 14 and 15 illustrate significant resections with reconstructions being accomplished for extensive local bone disease. Les et al. *(19)* reviewed, retrospectively, 78 patients from three institutions with osseous RCC. Forty-one (53%) patients were treated by intralesional methods and internal fixation. Seventeen had significant local progression causing failure of the stabilization and required 14 further procedures including nine wide resections with reconstruction, three amputations, and two mass excisions. The second group consisted of 37 (47%) patients, who underwent wide resection with either wide margins or mar-

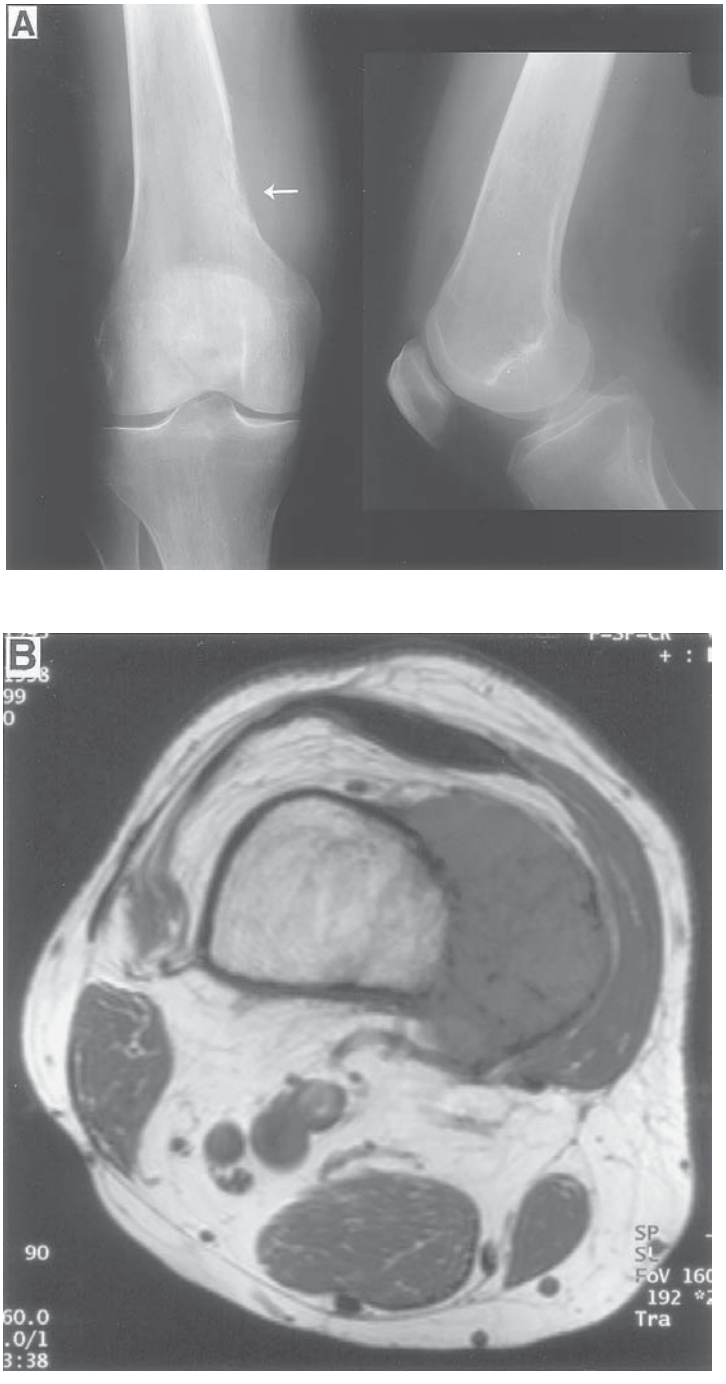


Fig. 10. (**A**) A 57-year old male with distal thigh pain. Radiographs show violation of the distal medial cortex. (**B**) MRI shows a huge external soft tissue mass on the medial side with little intramedullary involvement.

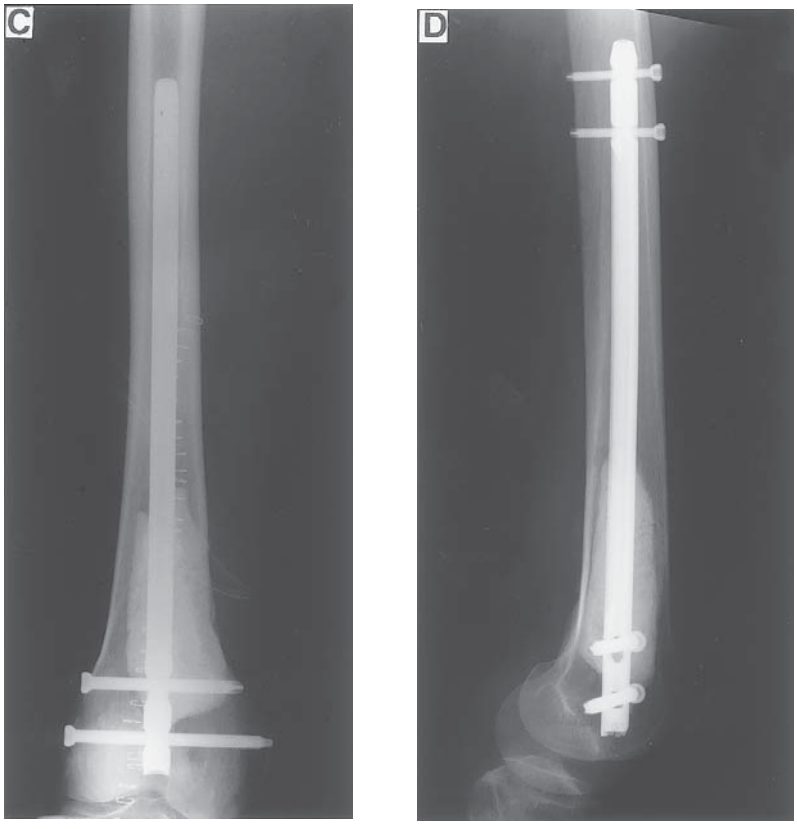


Fig. 10. (Continued). (**C**) and (**D**) Stabilization with a retrograde statically locked rod with supplemental methylmethacrylate immediately postoperative and at 6 mo. Case was done under tourniquet, the soft-tissue mass excised, no transfusions were required, and the patient received postoperative radiation therapy.

ginal margins with or without reconstruction. Only one further operative intervention for local bony progression in this group was needed. The Kaplan-Meier curves revealed that solitary metastases, longer disease-free interval from initial tumor diagnosis to the development of metastasis, and surgical resection in contrast to intralesional procedures were associated with a significantly improved survival. Based on the data, the conclusion from the paper recommended that resection surgery should be considered for renal cell skeletal metastasis when possible in order to lessen the risk of reoperation for local progression. Even though the patients with intralesional procedures had shorter survival, these patients who underwent intralesional orthopedic surgical intervention had a high risk of reoperation.

11. SUMMARY

The management of recognized metastatic osseous lesions of RCC is a team approach among the urologist, medical oncologist, radiation therapist, and the orthopedic surgeon. Appropriate aggressive approaches can improve patient quality of life and longevity. On occasion, treatment of the solitary osseous metastatic lesion as a primary tumor with an en bloc resection may yield a long-term survivor. Management of osseous lesions warrant the concept of using rods and cement in contrast to plates and screws to lessen early

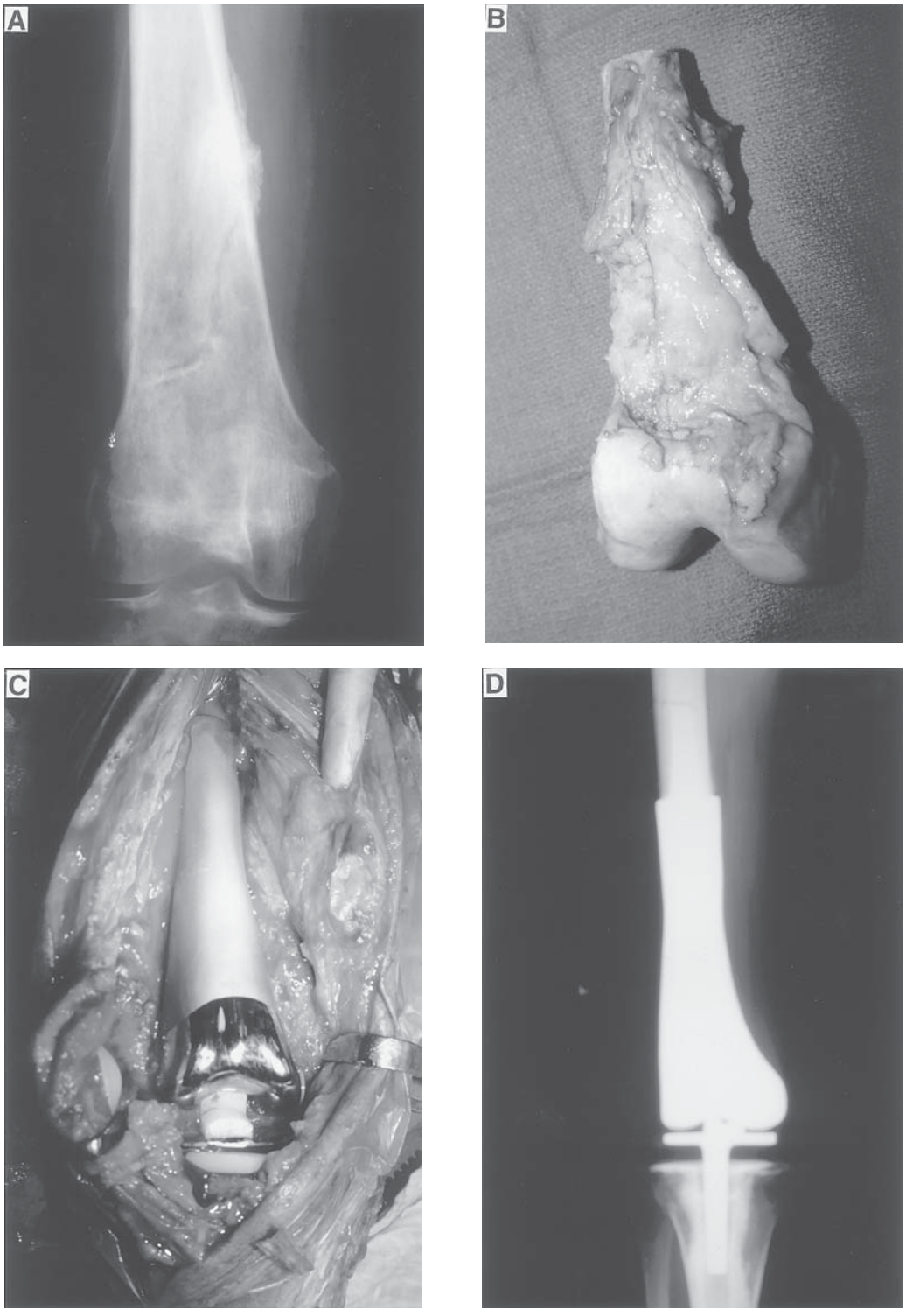


Fig. 11. (**A**) Painful distal femoral lesion in a 58-yr-old male after radiation therapy with an occult pathologic fracture trying to heal. (**B**) Resected specimen distal femur. (**C**) Rotating hinge modular total knee replacement intraoperatively. (**D**) Radiograph AP of rotating hinge femoral replacement at 6 mo.

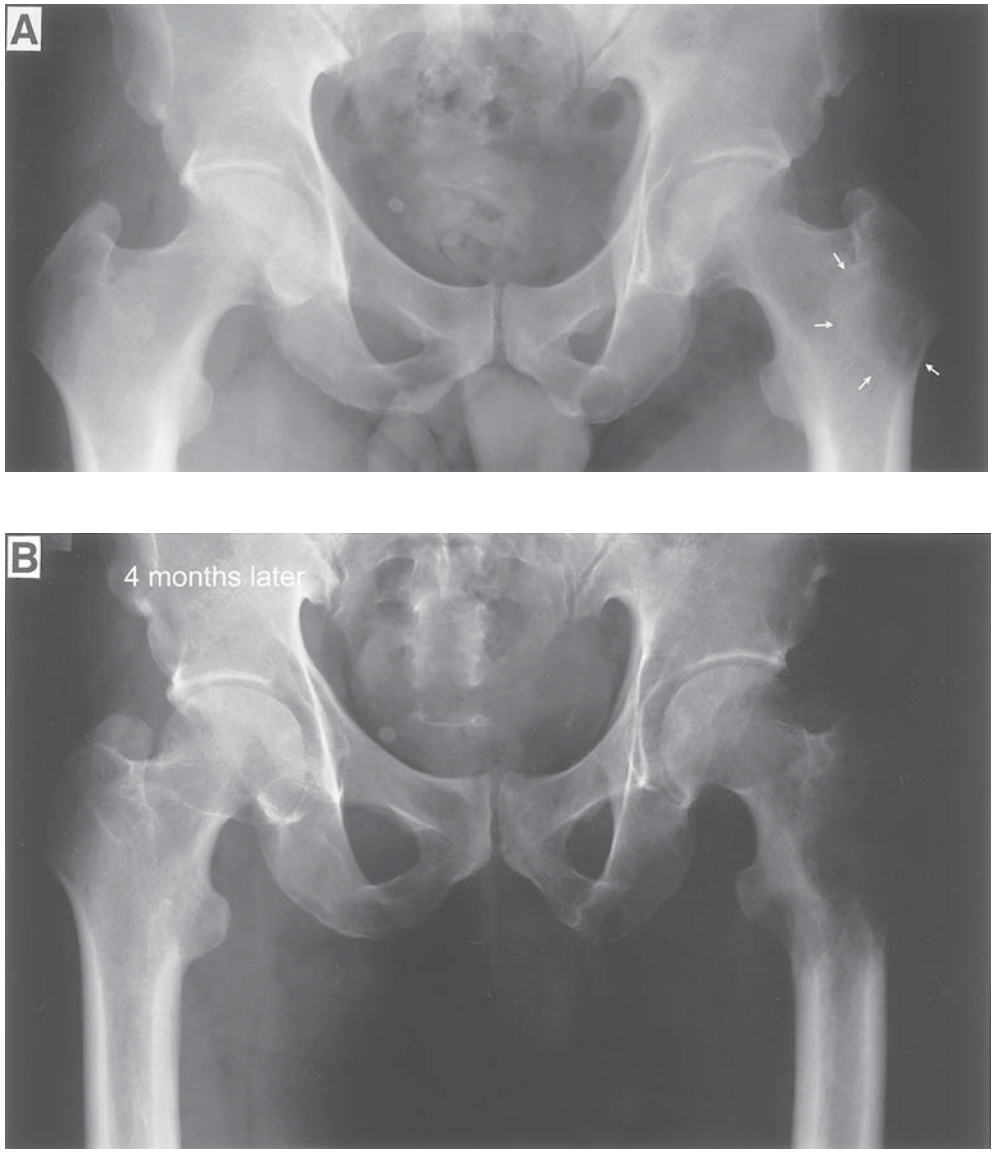


Fig. 12. (**A**) Mildly uncomfortable lytic lesion at the base of left greater trochanter in 57-yr-old male who had a nephrectomy 12 mo previously. (**B**) Painful huge destructive lesion with loss of bone integrity proximal femur within a four-mo interval of no treatment.

fracture fixation failure. In many instances, resection of the poor quality bone with subsequent replacement using limb salvage techniques will yield improvement in quality of life and a much reduced rate of failure of the construct. Early recognition of spine lesions with appropriate intervention will enhance overall function and reduce the incidence of neurologic functional loss. Improved function and pain relief using current orthopedic surgical techniques are goals. Application of orthopedic surgical oncology techniques by speciality trained surgeons with expertise and experience dealing with these complex problems will provide improved function, a better quality of life, and increased longevity for these patients.

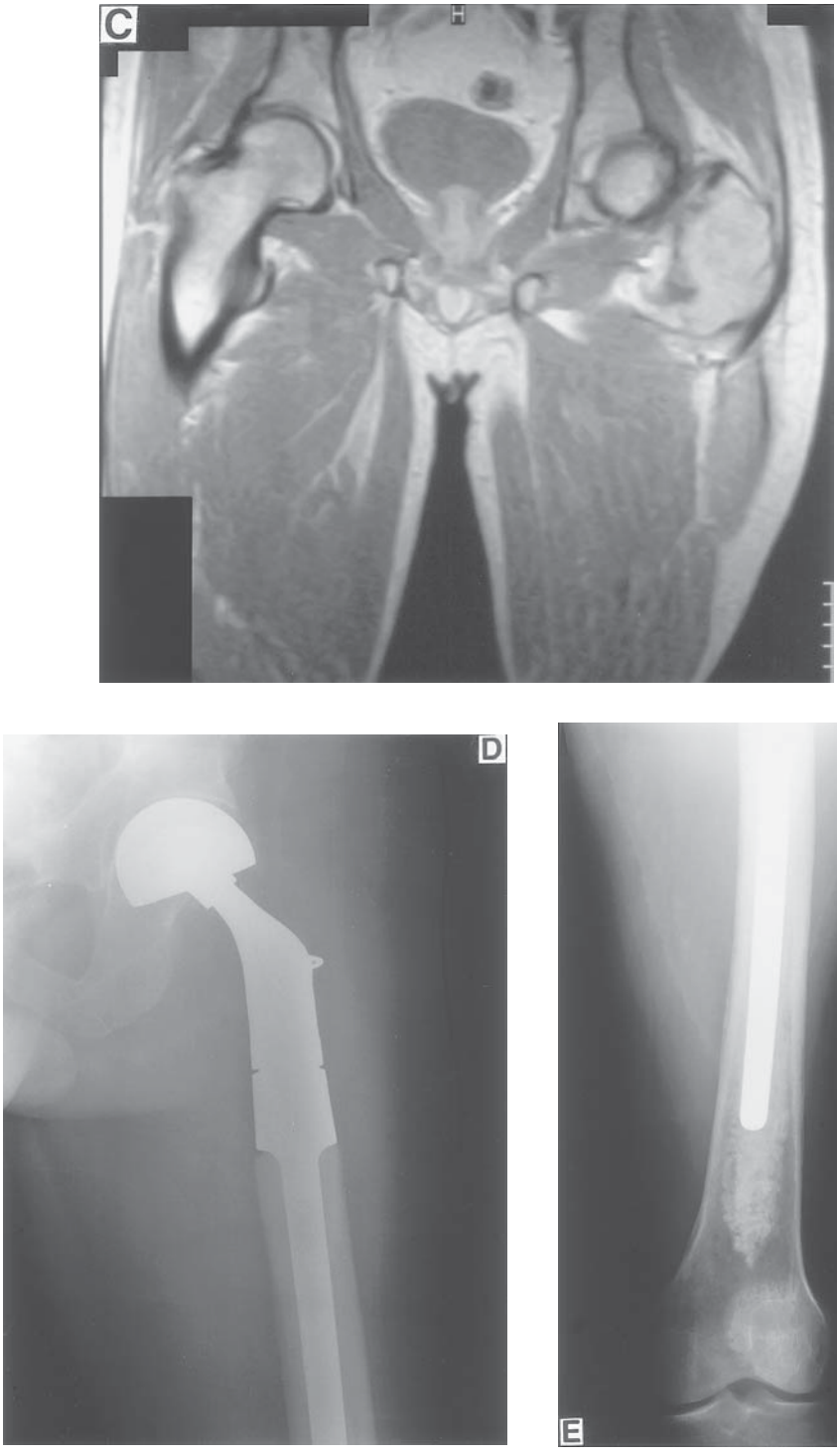
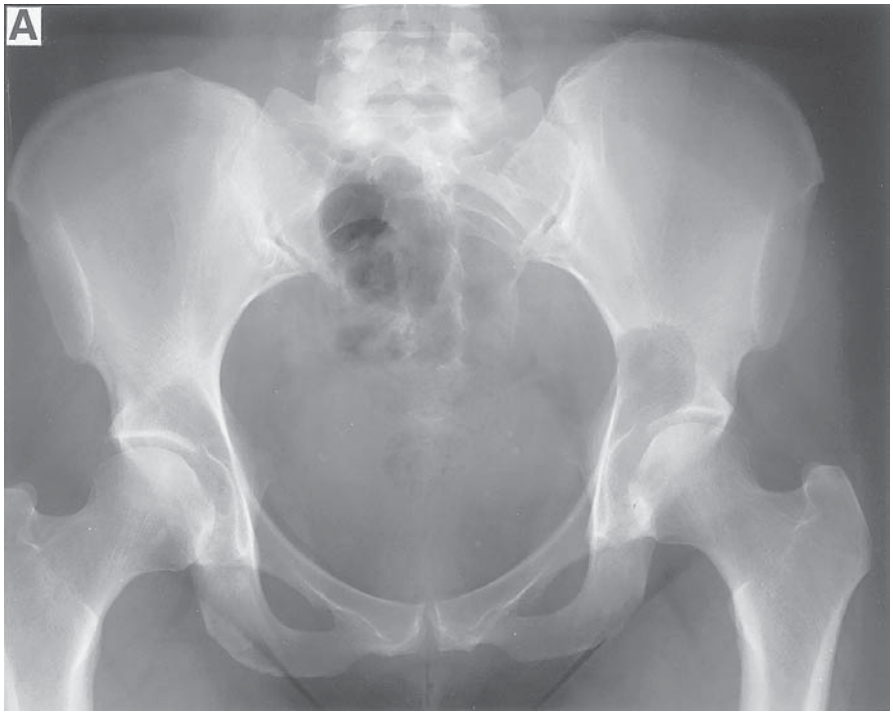


Fig. 12. (Continued). (**C**) MRI showing large soft tissue replacement mass. (**D**) and (**E**) Proximal femoral bipolar replacement with gluteus medius attachment through radiolucent broad synthetic suture to the component.



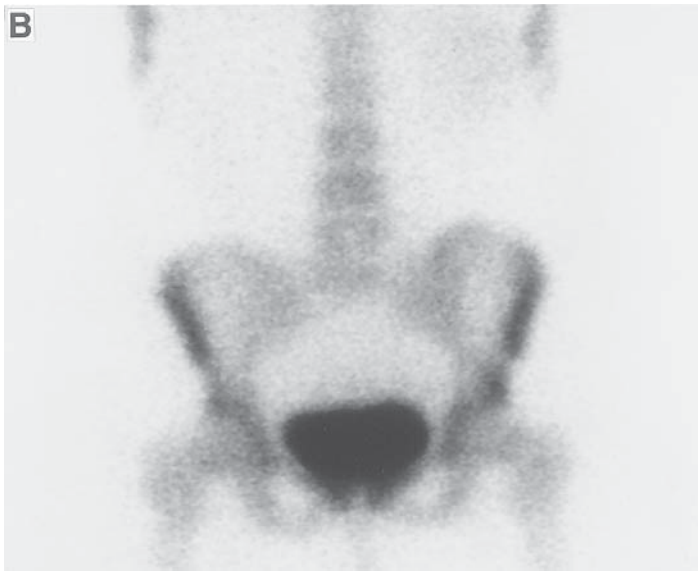
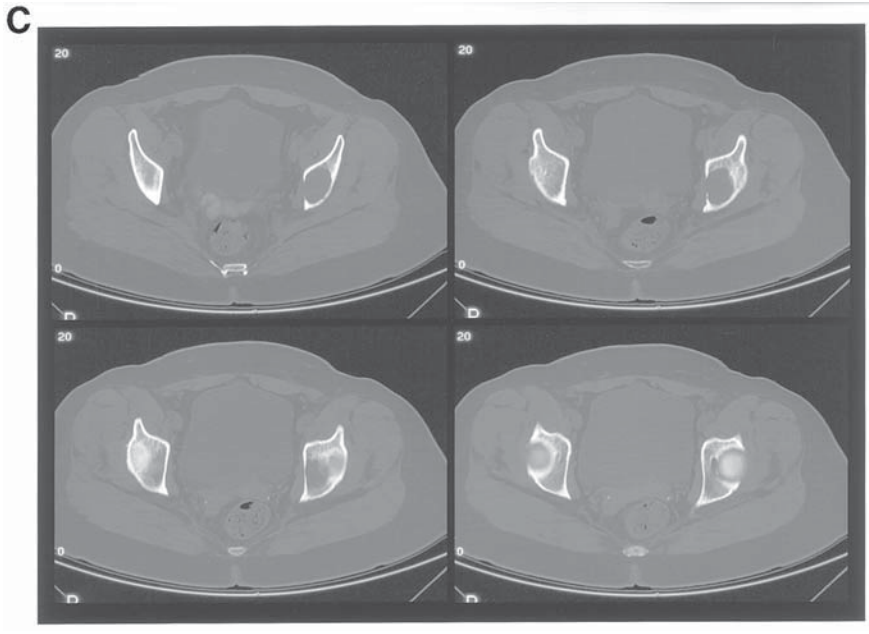


Fig. 13. (**A**) 48-yr-old female 2 yr after nephrectomy now with a solitary metastasis left supra acetabular area. (**B**) Bone scan shows only some increased uptake above the acetabulum.



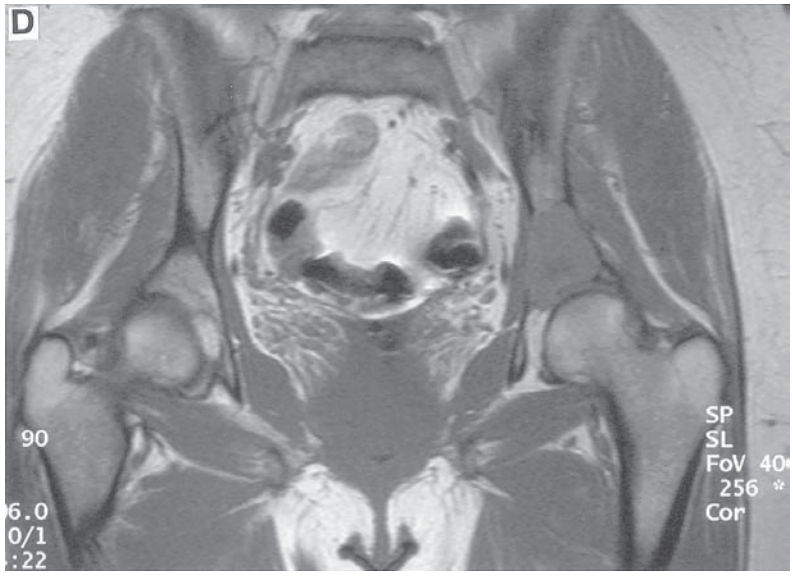


Fig. 13. (Continued). (**C**) CT scan shows involvement into the acetabular fovea. (**D**) MRI shows the soft-tissue mass and truly defines the intraosseous extent of the metastatic lesion.

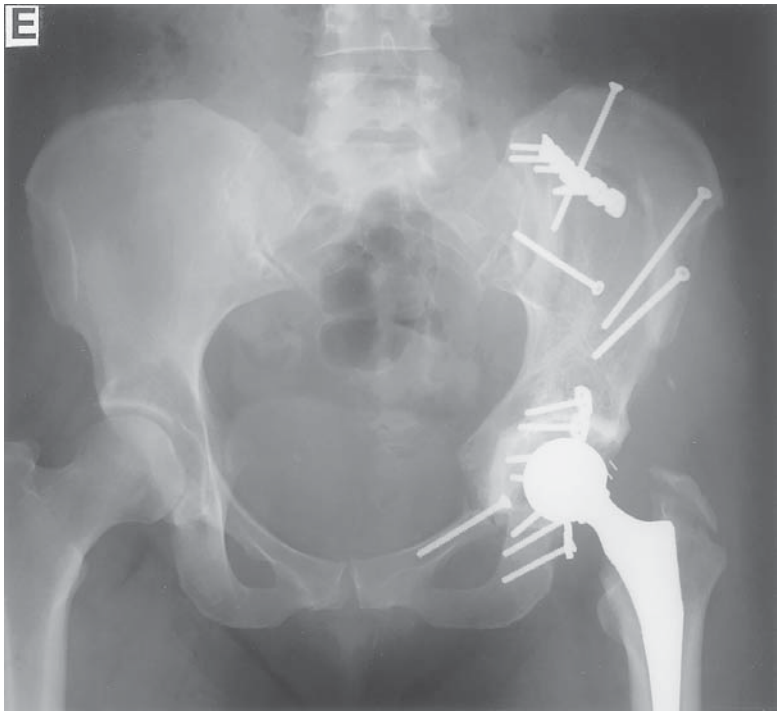


Fig. 13. (Continued). (**E**) Reconstruction with pelvic allograft and total hip replacement at 7 mo with the patient remaining disease-free.

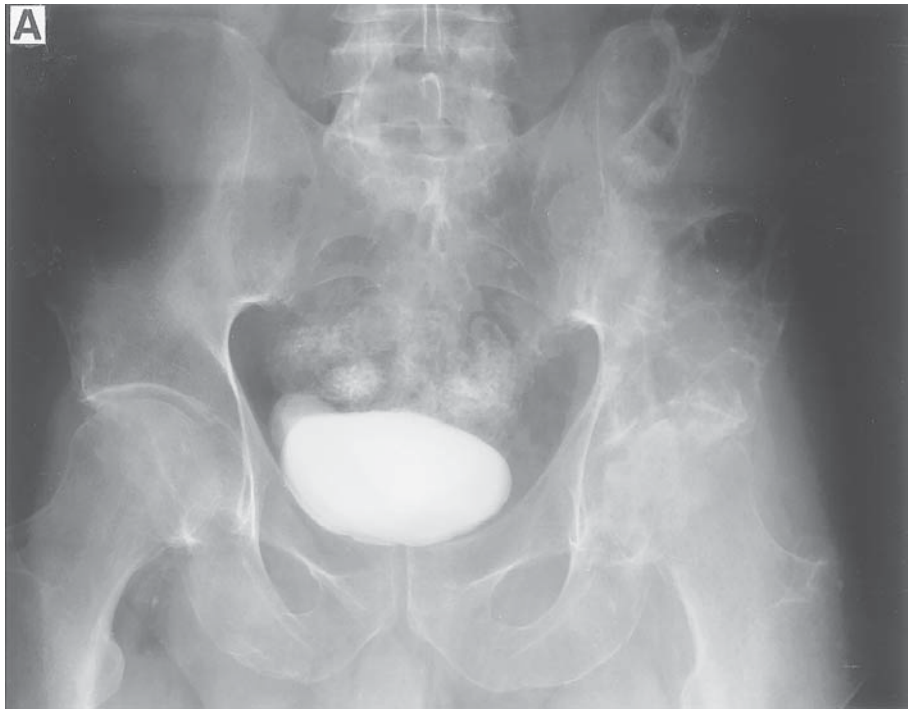


Fig. 14. (**A**) 64-yr-old male with a huge painful destructive renal cell lesion left pelvis. He has known other osseous sites, but has been a good responder to therapy.

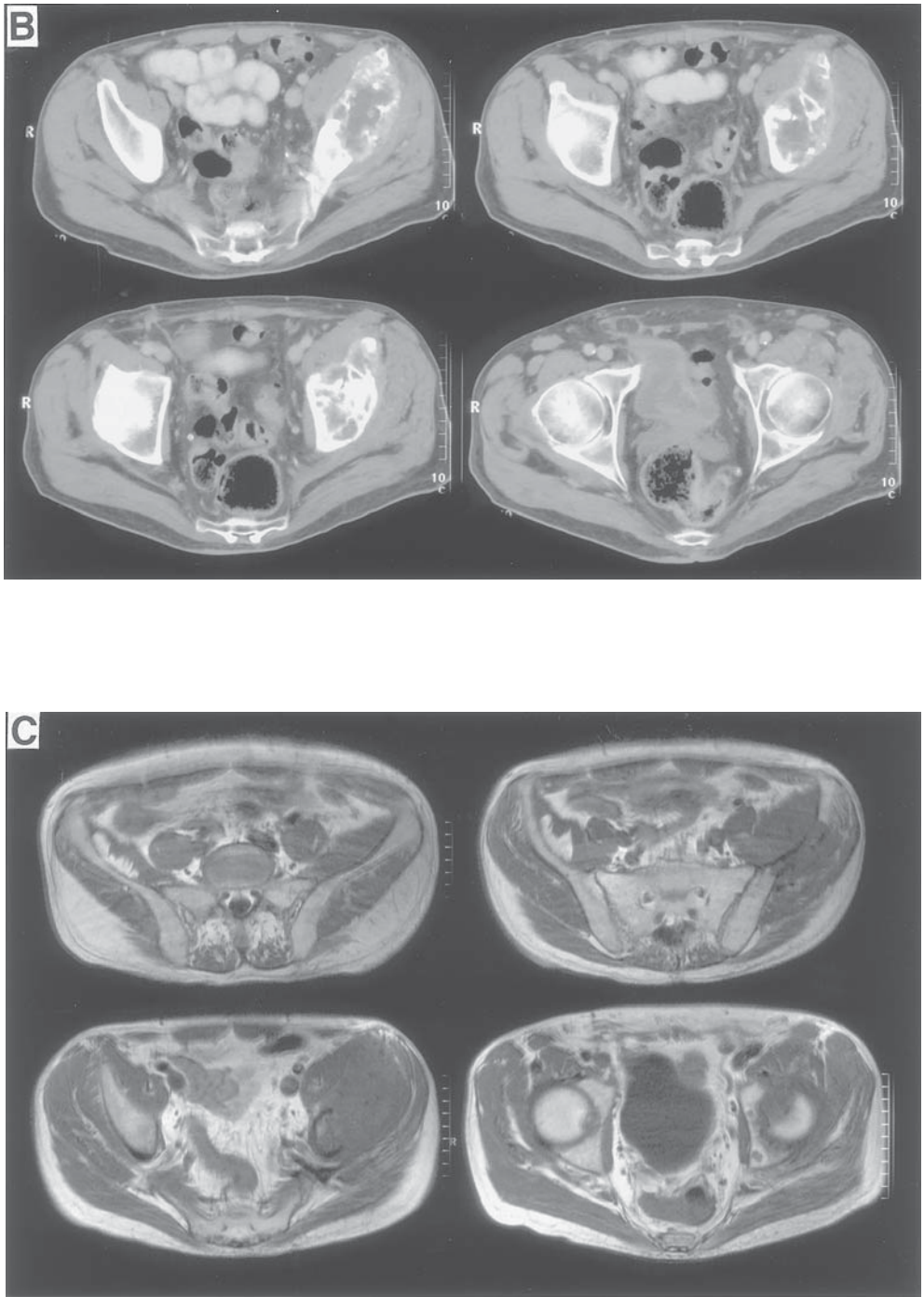


Fig. 14. (Continued). (**B**) CT scan showing a destructive pelvic lesion that involves the dome of the hip joint. (**C**) Large soft-tissue mass with involvement of the hip joint being quite evident on MRI.

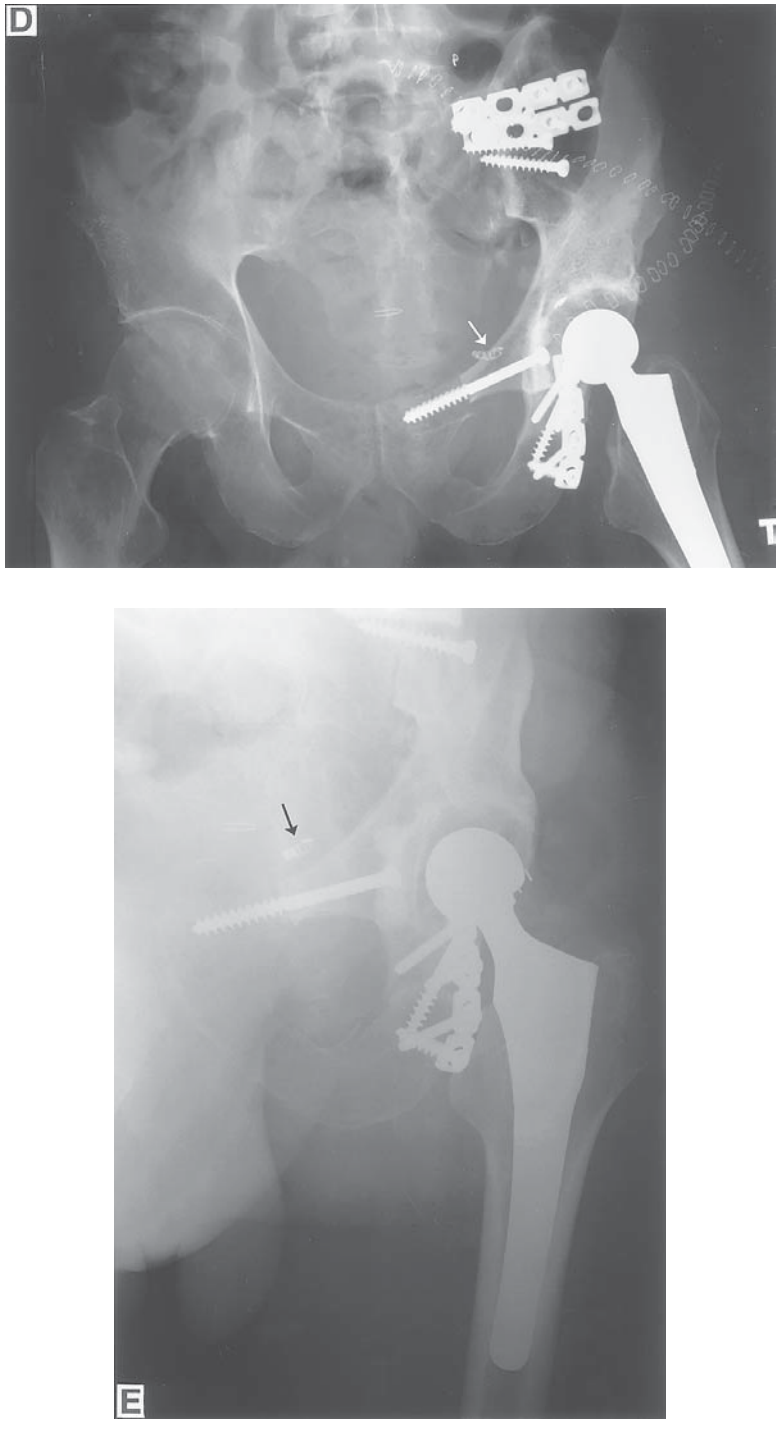


Fig. 14. (Continued). (**D**) Postoperative radiograph. Arrows point to embolization coils placed preoperatively. (**E**) Radiograph at 14 mo with patient ambulatory with a cane, but significant limp secondary to loss of gluteus medius from resection of involved tumor mass.

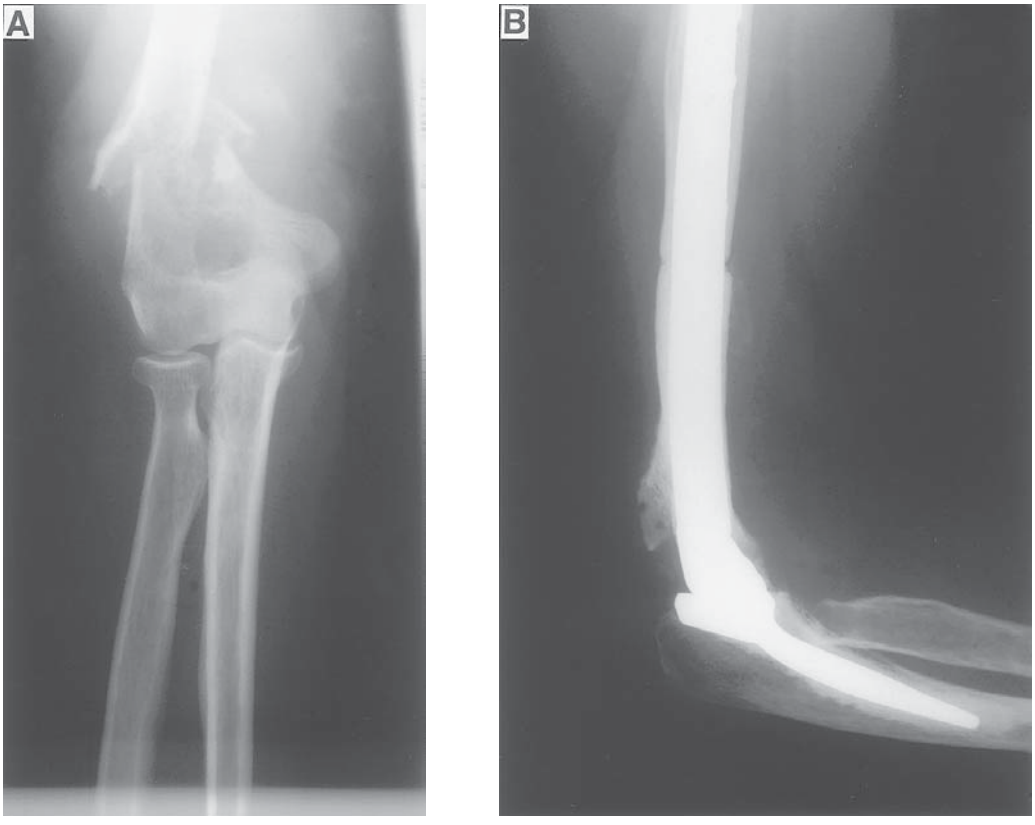


Fig. 15. (**A**) Fracture of distal humerus in 50-yr-old female. Patient was found to have a hypernephroma and underwent nephrectomy. (**B**) Management of this solitary lesion was resection with a long stem total elbow replacement over an allograft. She developed other osseous lesions after 2 yr.

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17 Management of Central Nervous System Metastases in Renal Cell Carcinoma Patients

John H. Suh and Gene H. Barnett

CONTENTS

1. INTRODUCTION

Brain metastases represent the most frequent neurologic complication of cancer and far outnumber the cases of primary brain tumors. An estimated 20–40% of cancer patients will develop brain metastasis during their lifetime with approximately 170,000 cases diagnosed per year *(1,2)*. Tumor types most likely to metastasize to the brain include lung, breast, melanoma, and renal carcinomas with lung cancer causing most cases. Melanoma has the highest propensity for brain metastases relative to other sites. Multiple metastases occur in up to 70% of cases with breast, renal, and colorectal cancer more likely to have a single lesion *(3,4)*. Eighty percent are supratentorial in location. Patients with brain metastases may succumb to systemic disease, neurologic causes, or a combination of both. Although the percentage of patients who develop brain metastases from renal cell carcinoma (RCC) is low (4–13%), this patient group represents an important area of ongoing investigation *(5,6)*.

Advances in radiation oncology, surgery, and imaging have led to earlier diagnosis, better treatments, and improved results for select patients. Given the multiple available treatment options for these patients, optimal management is controversial and evolving. This chapter will provide an overview of management for patients with brain metastases, in particular, RCC.

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2. SIGNS AND SYMPTOMS

RCC spreads to the brain by way of a hematogenous route. Once the tumor enters the brain, few barriers exist to its spread. Infiltration of different parts of the brain can lead to a variety of neurologic symptoms such as headaches, impaired cognition, seizures, personality changes, and motor weakness. These symptoms are related to the area of brain involvement. On occasion, the tumor can involve the leptomeningeal surface or cerebrospinal space. To help guide treatment, it is important to perform a thorough history and neurologic exam for patients with brain metastases.

3. IMAGING

Prior to the advent of modern imaging, the diagnosis of brain metastases was made mostly on history and physical exam findings. The use of pneumoencephalography and angiography helped aid diagnosis. The development of the CT scan in the 1970s and the MRI scan in the 1980s helped lead to earlier diagnosis and improved treatment of brain metastases. It is estimated that 50% of patients will have a single lesion on CT scan *(7)*. Fig. 1 demonstrates typical CT findings. With an MRI scan, fewer than 30% of patients will have a single lesion *(8)*. An MRI scan of the brain with and without gadolinium is the most sensitive test when evaluating patients with brain metastases (Fig. 2). The edema noted on T2 weighted and flair images is a result of increased brain water and ions arising from direct injury to the cells or from injury to the vascular endothelium *(9,10)*. Fig. 3 demonstrates edema from a large frontal metastasis. The differential diagnoses for brain metastases are listed in Table 1.

4. PROGNOSTIC FACTORS

Multiple prognostic factors have been analyzed for patients with brain metastases. These include number and size of lesions, location (deep vs superficial and eloquent vs noneloquent cortex), neurologic deficits, radiosensitivity of the tumor, time to development of brain metastases, systemic and primary disease status, general health, age, and Karnofsky performance status (KPS). Thus, work-up including CT chest, abdomen, and pelvis, bone scan, and laboratory tests should be obtained to determine extent of disease. Historically, RCCs have been considered radioresistant along with sarcomas and melanomas.

The Radiation Therapy Oncology Group (RTOG) reviewed the results of 1200 patients from three consecutive RTOG trials conducted between 1979–1993 using recursive partition analysis, a statistical method that creates a regression tree according to prognostic significance *(11)*.

Eighteen pretreatment characteristics and three treatment-related variables were analyzed. Based on these results, the RTOG suggested three classes: Class I (median survival 7.1 mo)—patients with KPS $\oplus$ 70, age < 65 yr old, controlled primary and no extracranial metastasis, Class II (median survival 4.2 mo)—patients $\oplus$ 65 yr old or uncontrolled primary and/or extracranial metastasis, and Class III (median survival 2.3 mo)—patients with KPS $\leq$ 60.

5. TREATMENT

Advances in imaging, surgery, and radiation therapy have greatly increased the treatment options for patients with brain metastases. Based on patient and tumor characteristics, numerous treatment options exist. Some of the currently used options are listed in Table 2.

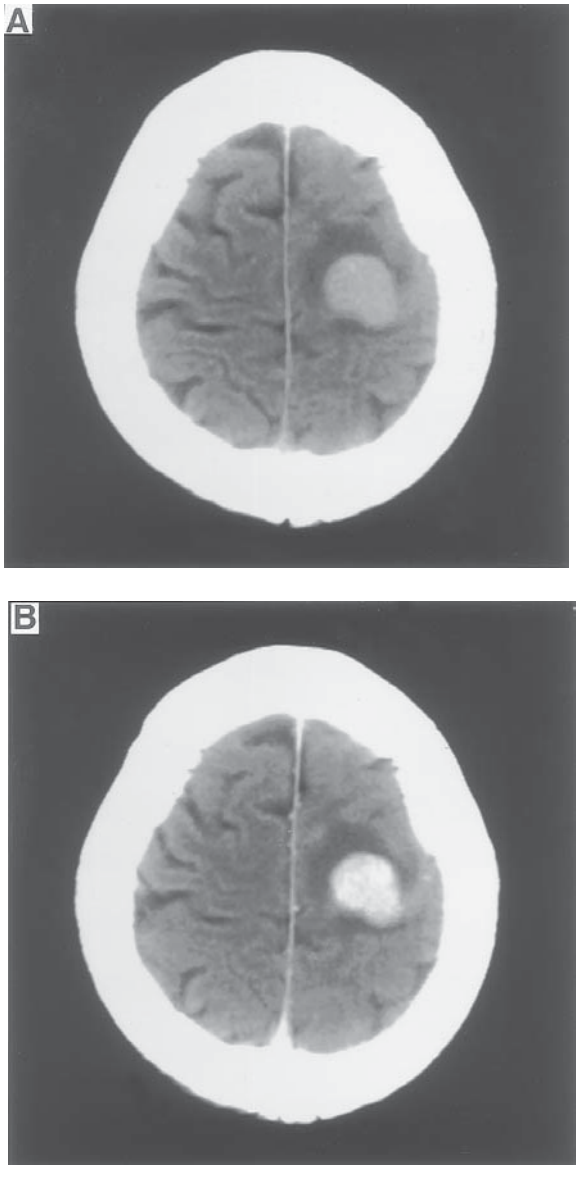

Fig. 1. CT of the brain with (**A**) and without (**B**) contrast of a single lesion in the frontoparietal region of a 71-yr-old woman with RCC.

5.1. Medical

The median survival for untreated patients is approximately 1 mo *(12)*. With corticosteroids, the survival is doubled to 2 mo *(13)*. Corticosteroids usually have a rapid effect on edema. In 60% to 80% of the time, steroids can resolve or reduce clinical symptoms *(14,15)*. Because this medication can have multiple side effects, such as weight gain and myopathy, it must be used with caution. The use of chemotherapy has been very limited and its role has not been clearly defined.

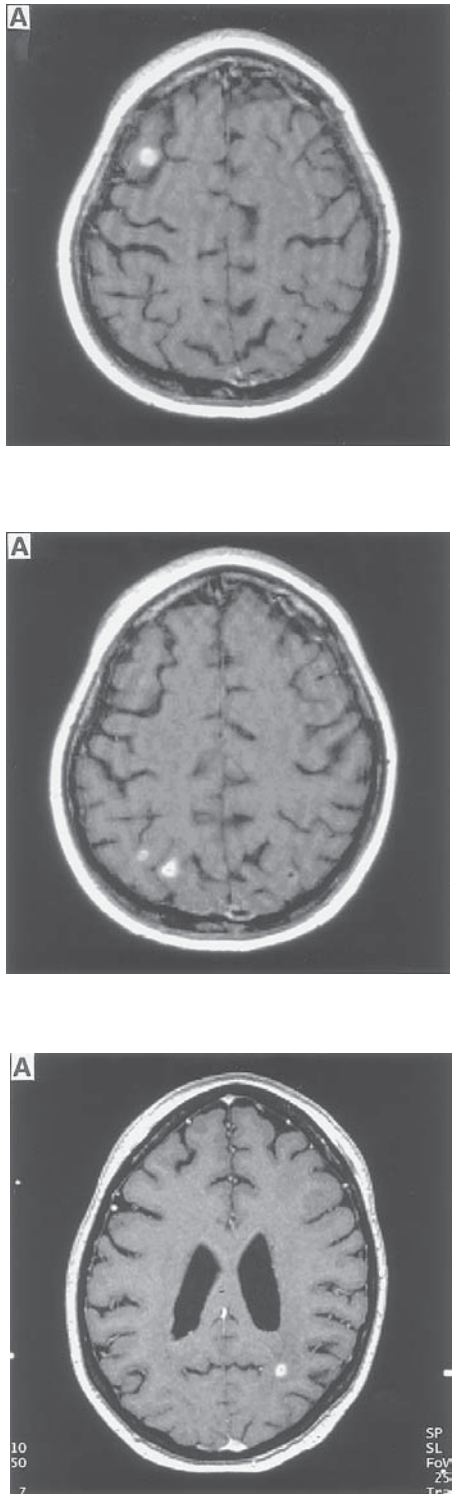

Fig. 2. MRI scan of the brain with Gadolinium (T1 images) from a 38-yr-old woman with multiple brain metastases from RCC.

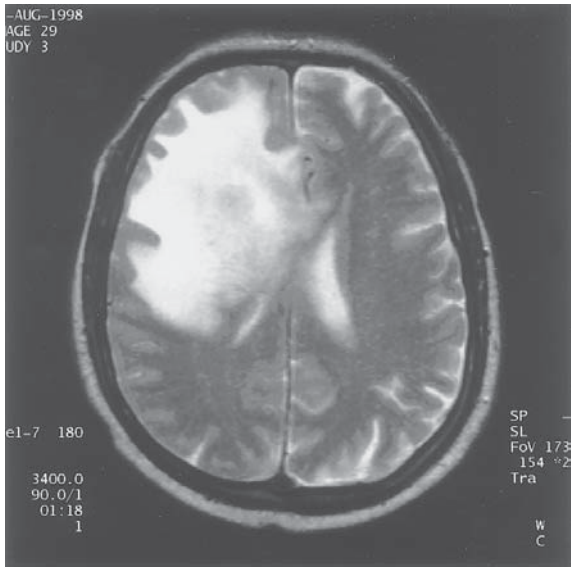

Fig. 3. MRI scan of the brain (T2 images) demonstrating edema from a large right frontal metastasis.

Table 1
Differential Diagnoses for Brain Metastases

1. Meningioma
2. Vascular malformation
3. Abscess
4. Glioma
5. Lymphoma
6. Granuloma
7. Paraneoplastic syndromes
8. Encephalitis

Table 2
Current Treatment Options for Patients with Brain Metastasis

1. Supportive care (steroids alone)
2. Whole brain radiation therapy
3. Surgery and whole brain radiation therapy
4. Surgery alone
5. Stereotactic radiosurgery and whole brain radiation therapy
6. Stereotactic radiosurgery alone
7. Chemotherapy

5.2. Whole Brain Radiation Therapy

Whole brain radiation therapy (WBRT) has been considered the treatment of choice for most patients with brain metastases since the first published report in 1954 *(16)*. The goal of WBRT is to deliver a uniform dose of radiation to the entire brain in hope of controlling gross disease and minimizing the risk for microscopic spread. Results with WBRT

Table 3
Advantages of Surgery
Over Radiosurgery for Brain Metastases

1. Pathologic confirmation of malignancy
2. Rapid reversal of neurologic deficits
3. Decreased radiation necrosis risk
4. Durable local control

increase median survival to 3–6 mo *(17)*. The RTOG has performed several trials evaluating various fractionation schemes ranging from 2000 cGy/5 fractions to 5000 cGy/20 fractions with no difference in neurologic function, duration of response, survival, or time to progression *(17,18)*. Local failure occurred in up to 50% of patients. Some papers have demonstrated an advantage to dose escalation to final doses of 6000 to 7000 cGy *(19,20)*. Most institutions, including ours, use 3000 to 4000 cGy using 200 to 300 cGy/fraction. The most sensitive histologies to conventional fractionated radiation include small-cell lung cancer, germ-cell tumors, and lymphomas. Although RCCs are usually considered to be "more radioresistant" to these radiation treatments *(21,22)*, some have reported improvement of symptoms after conventional radiation *(23,24)*.

The acute and long-term effects of WBRT are generally mild and manageable. The acute side effects include alopecia, erythema, fatigue, somnolence, headaches, nausea, otitis, and vomiting. In general, most of the acute side effects except for alopecia and somnolence syndrome resolve within 4 wk. The long-term risks are generally not a significant issue given the short life expectancy of most patients. Side effects may include neurocognitive dysfunction, leukoencephalopathy, radiation necrosis, and brain atrophy *(25,26)*. A small percentage (10%) of long-term survivors (>12 mo) may develop dementia, ataxia, and urinary incontinence *(27)*.

The results for WBRT have been disappointing for RCC patients. Retrospective results from M.D. Anderson Cancer Center of 119 patients who underwent WBRT were poor *(28)*. Median survival from diagnoses of brain metastases was 4.4 mo for all patients. Those with multiple lesions had median survivals of 3.0 mo compared to 4.4 mo for patients with a single lesion ($p = 0.043$). The cause of death was neurologic in 76% of patients. No survival difference was observed for patients who were diagnosed with synchronous vs metachronous metastases. Survival rates at 6 mo and 1 yr were 16.8% and 5.9%, respectively.

5.3. Surgery

Advances in microsurgical techniques, functional mapping, intraoperative localization, cranial imaging, and computers have decreased risks associated with open craniotomy. Surgery offers pathologic diagnosis, rapid alleviation of neurologic symptoms, and increased local control through gross total resection. Preoperative MRI scans allow the neurosurgeon to determine the accessibilty and resectability of the lesion. In general, lesions located in the brainstem, thalamus, and basal ganglia are not considered amenable to surgery. Most patients selected for surgery have life expectancies of greater than 4–6 mo. The potential advantages of surgery are listed in Table 3.

Two prospective, randomized trials have demonstrated the value of surgical resection for metastases followed by WBRT vs WBRT alone. Forty-eight patients from the University of Kentucky were treated with biopsy and WBRT or complete surgical resection

and WBRT *(29)*. The surgery and WBRT provided statistically better results in terms of local control, neurologic function, and survival. Another randomized trial from the Netherlands evaluated 63 patients and demonstrated superior survival for the surgery and WBRT group (10 mo) vs WBRT alone group (6 mo) *(30)*. However, a Canadian multicenter randomized trial did not demonstrate a difference in quality of life or survival between patients undergoing surgery and WBRT vs WBRT alone *(31)*.

For patients with multiple lesions, the use of surgical resection has historically not been advised. Bindal et al. has, however, suggested selected patients with multiple metastases that are completely resected can achieve survivals comparable to single lesions (median survival of 14 mo) *(32)*. Generally, patients with more than three lesions are not considered good candidates for surgery.

To address the value of WBRT for patients with single brain metastasis resected surgically, Patchell and colleagues reported results of a prospective, randomized trial of 95 patients with brain metastasis *(33)*. Randomization was no further therapy vs WBRT. Statistically, significant results favoring the WBRT arm were noted in local recurrence, development of other brain metastases, and neurologic death. No difference in survival or functional independence was noted. They concluded surgery and WBRT results in fewer brain recurrences and decreases the chance of dying from neurologic causes.

The largest surgical series of RCC patients was reported by Wronski and colleagues *(34)*. They published the results of 50 patients with RCC who underwent surgical resection from 1974–1993. Eighteen percent had multiple metastases. Median survival was 12.6 mo from craniotomy. Postoperative mortality was 10% with 46% having intratumoral hemorrhage. Postoperative complication rate was 28%. Cerebellar lesions did worse (median survival 3.0 mo). Twenty-two patients received WBRT and 18 did not receive WBRT. No survival difference was noted. One-yr and 5-yr survivals were 51% and 8.5%, respectively.

6. STEREOTACTIC RADIOSURGERY

Stereotactic radiosurgery (SRS) delivers a large single dose of highly focal radiation to a small, well-defined intracranial target. SRS uses multiple converging beams of radiation to develop a sharp-dose gradient that is characteristic of this type of radiation. This allows for the safe delivery of a high dose of radiation. This concept was first described by a Swedish neurosurgeon, Lars Leksell, and combined advances in stereotactic localization with use of a radiation source *(35)*. Originally, it was used for functional disorders, such as trigeminal neuralgia and psychiatric conditions. Malignant tumors were not considered good targets for SRS because of their large size and invasiveness. The introduction of the CT and MRI scans in the 1970s and 1980s facilitated SRS delivery and allowed for treatment of malignant tumors.

Three different technologies are being used to perform SRS. Each of these technologies use multiple convergent beams of radiation to deliver a large amount of radiation to a defined target, while minimizing dose to the normal surrounding tissues. The Gamma Knife (Elekta Corp, Stockholm, Sweden) uses up to 201 Co-60 sources arranged in a hemispheric distribution around the patient's skull. Linear accelerator-based systems (LINAC) use multiple arcs of radiation using photons and various table positions to achieve the steep-dose gradient. The cyclotron or synchrocyclotron uses beams of charged particles, typically protons.

Although there are several methods of SRS delivery, the steps to achieve the sharp-dose gradients are similar. A head frame is placed under local anesthesia to provide a fixed reference system. Following placement of the headframe, CT and MRI scans are performed to identify and define the tumor. These images are transferred to a computer where the tumor and normal structures are contoured. The neurosurgeon, radiation oncologist, and medical physicist devise a plan to maximize coverage and radiation delivery to the tumor, while minimizing dose to the normal tissues. Current planning systems allow rapid display of the three-dimensional target and isodose distribution on CT or MRI images. Fig. 4 shows a treatment plan for a Gamma Knife radiosurgery patient. Depending on the size, shape, and location of the tumor, multiple shots or targets may be used to deliver the most conformal radiation dose. For Gamma Knife radiosurgery, the frame is attached to one of four helmets, each having 201 collimators that direct the gamma rays to a focal target. Following treatment, the patient is usually discharged 1 h after treatment.

Brain metastases have characteristics that make them favorable for SRS. They are usually small at presentation (<3 cm), displace, rather than coexist, with normal brain tissue, are pseudospherical in shape, and are radiographically distinct targets. The lesions should be 5 to 10 mm away from critical structures, such as the optic chiasm. The goal with radiosurgery is to control the tumor while minimizing the risk for acute and long-term complications, such as radiation necrosis. The potential advantages of radiosurgery over surgery are listed in Table 4. Currently, brain metastases represent the most common indication for radiosurgery.

Historically, RCCs have been considered radioresistant tumors. However, results from the Joint Center for Radiation Therapy in Boston over a 7-yr period demonstrated no difference in local control rates between radiosensitive and radioresistant histologies treated with SRS with an overall actuarial local control rate of 85% at 1 yr *(36)*. Factors associated with decreased local control included recurrent tumor, large tumor volume, and infratentorial location. The median survival from date of SRS was 9.4 mo. The survival was equivalent for patients with one or two lesions, but significantly worse for those with three or more lesions. The Gamma Knife-users group also had similar local control and survival results *(33)*. Fig. 5 demonstrates pretreatment and posttreatment MRI scans for a man who underwent Gamma Knife radiosurgery at the Cleveland Clinic.

The largest reported radiosurgery experience for RCC patients is from Mori and colleagues *(38)*. They reviewed the results of 52 lesions from 35 consecutive RCC patients who underwent stereotactic radiosurgery at the University of Pittsburgh from 1988 to 1996. Eligibility included those patients with tumors less than 3.5 cm in greatest dimension and KPS ⊕ 50. Twenty-eight patients received whole brain radiation therapy as part of their management. Mean radiosurgery tumor margin dose was 1700 cGy (range 1300–2000 cGy). Median survival from date of radiosurgery was 11 mo. Neurologic death occurred in 12%. Local control was 90% with 21% achieving complete tumor regression. No delayed tumor hemorrhage was noted in any patients. The addition of WBRT to SRS did not appear to improve survival or prevent development of new remote tumors.

The use of WBRT especially for the radioresistant histologies, such as RCC is controversial. In Europe, it is not uncommon to omit WBRT. At the Karolinska Institute in Sweden, the local control rate with Gamma Knife alone is 94% *(39)*. Analysis of the Cleveland Clinic's overall single brain metastasis experience suggest that WBRT leads to better survival and freedom from progression *(40)*. Pirzkall et al. also reported improved

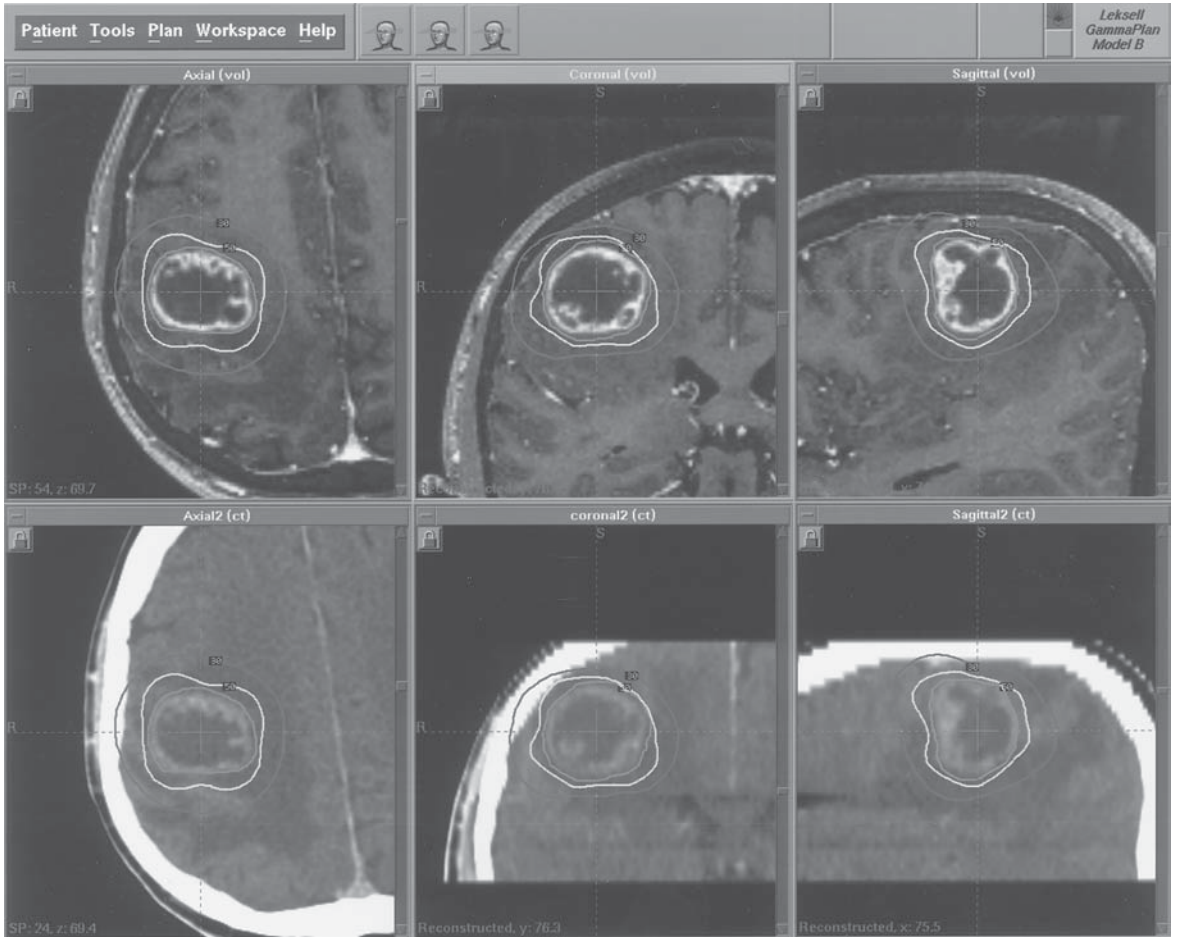

Fig. 4. Gamma Knife radiosurgery planning for a patient with metastasis to the right posterior frontal region using multiple targets (11 isocenters or shots). Serial axial, coronal, and sagittal images are shown in the upper (MRI) and lower (CT) panels. 1500 cCy prescribed to the 50% isodose line.

Table 4
Potential Advantages of Stereotactic Radiosurgery Over Craniotomy for Brain Metastases

1. Outpatient delivery
2. Local anesthesia
3. Minimal risk for bleeding or infection
4. Decreased costs
5. Minimal recovery time
6. Treatment of deep or eloquent areas of the brain
7. Treatment of multiple lesions

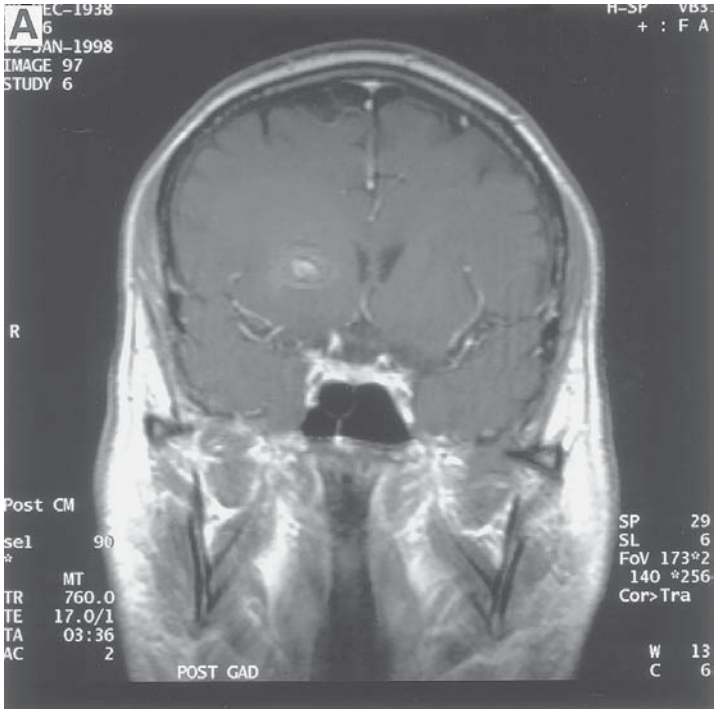

Fig. 5. (**A**) Pretreatment MRI scan of a 59-yr-old man with RCC metastatic to the right internal capsule.

median survival and local control especially in patients who underwent radiosurgery and WBRT vs radiosurgery alone *(41)*. The Eastern Cooperative Oncology Group is performing a phase I/II trial of stereotactic radiosurgery alone for patients with radioresistant histologies. The European Oncology Research and Treatment Consortium is currently performing a phase III trial of WBRT and SRS vs SRS alone.

The costs associated with SRS are less than surgery. Mehta reviewed the computerized billing records for all patients undergoing surgery and LINAC-based radiosurgery for brain metastasis *(42)*. Cost effectiveness, defined as the cost per year of survival, was evaluated. SRS offered superior cost outcomes on all measures. The average cost per week of survival was $310 for WBRT, $524 for surgery and WBRT, and $270 for WBRT and SRS. A study from the University of Pittsburgh compared surgery vs Gamma Knife radiosurgery and demonstrated the cost effectiveness of the Gamma Knife *(43)*.

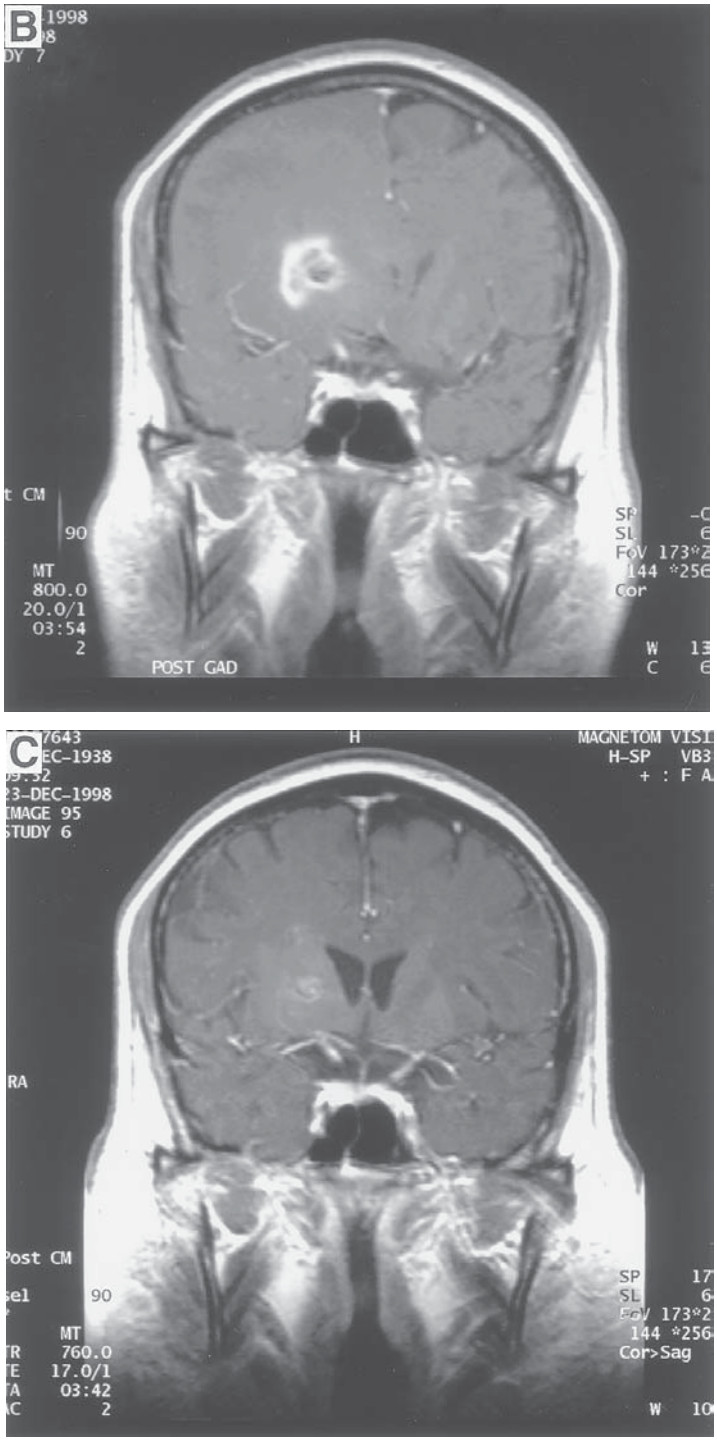

Fig. 5. (Continued). (**B**). Posttreatment MRI after Gamma Knife radiosurgery alone (2500 cGy prescribed to the 72% isodose line). Scan demonstrates radiation necrosis with midline shift and compression of the ventricles (7 mo status post Gamma Knife radiosurgery). (**C**) Scan demonstrates resolution of radiation necrosis with decreased tumor size and ventricular compression after 6 wk of steroids. Patient's Karnofsky performance status at follow-up was 90.

7. DISCUSSION

Advances in imaging, surgery, and radiation therapy have greatly increased the therapeutic options and have challenged the traditional therapeutic paradigms for brain metastasis patients. Thus, the management of brain metastases is controversial especially for a single, surgically accessible lesion. It appears that more aggressive management of these patients is advantageous and leads to better survival and quality of life. Even for patients with multiple lesions, aggressive management appears beneficial for selected patients. Because treatment is based on a number of factors, treatment needs to be individualized and should be based on the patient's overall condition.

Because radiosurgery is minimally invasive, is an out-patient procedure, and can treat surgically inaccessible lesions, some have advocated that it should be used *instead* of surgery for small (<3.5 cm) asymptomatic lesions. Two retrospective trials compared both modalities with opposite conclusions *(44,45)*. No randomized trial has compared radiosurgery to surgery in managing these patients. Institutions have attempted to randomize patients to such a trial with poor accrual secondary to patient or physician bias towards either surgery or radiosurgery *(46)*.

Historically, WBRT has been a mainstay for patients with brain metastases. With improved treatment options for these patients, some have challenged the need to irradiate all patients with brain metastasis given the concern of long-term toxicity. For patients with survivals greater than 1 yr, some have recommended either more protracted courses of radiation to minimize side effects or omission of radiation. Several trials are currently ongoing to determine whether radiosurgery alone is sufficient treatment for selected patients with brain metastasis.

Another controversy is whether stereotactic radiosurgery and WBRT is better than WBRT alone in terms of local control and survival. The Radiation Therapy Oncology Group is performing a prospective, randomized trial comparing WBRT vs WBRT followed by a radiosurgery boost.

Appropriate treatment options are best achieved when clinical decisions are made through a multidisciplinary team of neurosurgeons, radiation oncologists, medical oncologists, and neurooncologists. Since the best treatment option remains controversial, the potential benefits and risks should be carefully explained to the patient and family. Quality-of-life issues have not been fully explored for patients with brain metastases and will undoubtedly play a major role in future treatment recommendations. Current and future trials will continue to guide treatment options and will allow patients and physicians to make prudent decisions regarding treatment.

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18 Chemotherapy for Metastatic Renal Cell Carcinoma (RCC)

Javier A. Ruiz, Haralambos Raftopoulos, and Daniel P. Petrylak

CONTENTS

1. INTRODUCTION

In 1998, it was estimated that 29,900 cases (male: 17,600 and female: 12,300) of kidney cancer were diagnosed in the United States, with 11,600 patients (male: 7100 and female: 4500) succumbing to metastatic disease *(1)*. The treatment of metastatic renal cell carcinoma remains a challenge. Although modest responses to immunotherapy with interleukin-2 (IL-2) and interferon (INF) can be observed in with metastatic disease, cytotoxic therapy is ineffective. The last comprehensive review of this subject by Yagoda et al. in 1995 found an overall response rate of 6% in 4093 adequately treated patients with advanced renal cell carcinoma (RCC). A slight improvement in this response rate to 14.6% was found in patients treated with Floxuridine or 5-fluorouracil *(2)*.

To determine whether improvements in cytotoxic therapy for RCC has been achieved since 1995, single and multiagent phase II (Table 1) and III trials (Table 2) published between 1993 to 1998, were reviewed, including studies reported in abstract form. A total of 2327 patients were entered in these studies. To provide a basis for accurate comparison, studies were reviewed using the same criteria for prior treatment, response, dose escalation, and calculation of response rate as in the prior review by Yagoda. Prior immunotherapy was not considered to be prior treatment; patients were considered as pretreated only if they received prior chemotherapy. Although all studies consistently define complete response (CR) as the disappearance of all disease and partial response (PR) as a >50% reduction of measurable disease, the categories of minor response (MR), and stable disease (SD) are somewhat less well defined and are not consistently reported.

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Table 1
Phase II trials

Reference(s)	*No. Entered*	*No. Adequate*	*No. Inadequate*	*No. prior chemo-therapy*	*No.* *CR*	*PR*	*Percent of Adequate CR + PR (95% CI)*	*MR/ STA B*	*Initial (highest) Dose*
Amonafide									
Witte et al. *(3)*	19	17	2	19	0	0	0 (0–23)	[a]	Amonafide 300 mg/m^2/d IV over 1 h X 5 days Q 21 days
Caracemide									
Witte et al. *(3)*	18	17	1	18	0	0	0 (0–23)	[a]	Caracemide 550 mg/m^2/d IV as a 16 h fusion X 5 days Q 21 days
Coumarin and Cimetidine									
Marshall et al. *(4)*	37	31	6	35	2	4	19 (8–38)	[a] / [a]	Coumarin 400 mg (7 gm) po qday and cimetidine 300 mg po QID starting on D 15.
Docetaxel									
Bruntsch et al. *(5)*	32	27	5	32	0	1	4 (0–21)	[a] /9	Docetaxel 100 mg/m^2 IV Q 3 wk
Doxorubicin (liposomal, encapsulated-LED)									
Law et al. *(6)*	14	14	0	[a]	0	0	0 (0–27)	[a]	LED 75 (105) mg/m^2/d IV over 1 h Q 21 days
Pennington et al. *(7)*	32	28	4	32	0	1	4 (0–20)	[a] /13	Liposomal doxorubicin 50 mg/m^2 IV Q 28 days
Doxorubicin + Vinblastine + Tamoxifen + Quinine									
Ravaud et al. *(8)*	17	[a]	[a]	[a]	0	0	0 (0–23)	[a]	Doxorubicin 50 mg/m^2 IV + Vinblastine 5 mg/m^2 IV Q 21 days; Tamoxifen 400 mg/m^2 po on D -1, 300 mg/m^2 on D1-12 + Quinine 30 mg/kg/d po on D-1 + D1.
Echinomycin									
Chang et al. *(9)*	17	13	4	17	0	0	0 (0–28)	[a]	Echinomycin 1200 mg/m^2 IV QW X 4 weeks Q 6 weeks
Marshall et al. *(10)*	49	47	1	[a]	0	1	2 (0–13)	[a]	Echinomycin 1.25 mg/m^2 IV Q 28 days

Edatrexate									
Dreicer et al. *(11)*	44	37	7	36	0	2	5 (1–20)	[a]/6	Edatrexate 80 mg/m^2 IV QW X 5 weeks
Floxuridine									
Wilkinson et al. *(12)*	29	29	0	[a]	1	5	21 (9–40)	[a]/13	Floxuridine 0.075 (0.275) mg/ kg/d CI IV X 14 days Q 28 days
Conroy et al. *(13)*	30	28	2	2[a]	0	4	14 (5–34)	3/5	Floxuridine 0.15 (0.2) mg/kg/d circadian-timed CI IV X 14 days Q month
Floxuridine + IFNa									
Chang et al. *(14)*	24	[a]	[a]	[a]	1	5	25[a] (11–47)	[a]	FUDR 0.075 ([a]) mg/kg/d CI X 14 D Q 28 D + IFNa-2b 3 MIU/m^2 TIW X 2 weeks, starting on the 2nd cycle.[a]
Falcone et al. *(15)*	42	39	3	25	3	10	33 (20–50)	3/15	FUDR 0.1 (0.2) mg/kg/d CI IV X 14 days q 28 days + IFNa-2b 10 MIU IM TIW
Floxuridine + IL-2									
Chang et al. *(14)*	15	[a]	[a]	[a]	0	4	27[a] (9–55)	[a]	FUDR 0.075 ([a]) mg/kg/d CI X 14 D Q 28 D + IL-2 5 MIU/m^2 Q Monday-Friday X 3 weeks, starting on the 2nd cycle.[a]
Floxuridine+ IL-2 + IFNa									
Gitlitz et al. *(16)*	31	23	8	[a]	0	6	26 (11–49)	[a]/[a]	5-FUDR 750 mg/m^2 CI IV D1-5 wk 1, IFNa 6 MIU/m^2 SC D 1 -4, wk 2-5 + IL-2 5 MIU/m^2/d CU UV D1-D4, wk 2–5 [a]
Floxuridine+ Vinblastine									
Small et al. *(17)*	15	14	1	15	0	4	29 (10–58)	[a]/5	Floxuridine 0.075 (0.225) mg/kg/d CI X 14 days, followed by Vinblastine 0.7 (0.9) mg/m^2/d X 14 days; cycle repeated Q 28 D
Fluorouracil+ IL-2+ IFNa									
Atzpodien et al. *(18)*	35	35	0	31	4	13	49 (32–66)	[a]/13	IL-2 20 MIU/m^2 SC TIW on wk 1+ 4 and 5 MIU/m^2 TIW on wks 2+3; IFNa 2 6 MIU/m^2 SC once weekly on wk 1 and 4, TIW on wks 2 + 3, and 9 MIU/m^2 TIW on wks 5–8 + 5-FU 750 mg/ m^2 IVb q wk on wks 5–8.
Sella et al. *(19)*	27	19	8	[a]	3	6	47 (25–71)	[a]/[a]	IFNa 4 MIU/m^2 SC QD; IL-2 2 MIU/m^2 IV + 5-FU 600 mg/m^2 IV, both CI daily X 5 days, repeated every 28 days.

Lopez et al. *(20)*	120	120	0	104	13	34	39 (31–49)	[a]/49	IL-2 20 MIU/m^2 SC TIW on wks 1+4, 5 MIU/m^2 TIW on wks 2+3; IFNa 2 6 MIU/m^2 SC QW on wk 1+4, then TIW on wk 2+3 and 9 MIU/m^2 TIW on wks 5–8 and 5-FU 750 mg/ m^2 IVb on week 5–8.
Dutcher et al. *(21)*	46	36	10	[a]	0	7	19 (9–37)	[a]/[a]	IFN 6 MIU/m^2 SC on D1 + IL-2 10 MIU/ m^2 SC BID on D 3–5: wk 1+4; IFN 6 MIU/m^2 SC + IL-2 5 MIU/m^2 SC on D 1, 3,5: wk 2,3; 5-FU 750 mg/m^2 IVb on D 1 + IFN 9 MIU/m^2 SC on D1,3,5: wks 5–8.[a]
Olencki et al. *(22)*	18	[a]	[a]	[a]	0	0	0 (0–22)	[a]/[a]	IL-2 5 MIU/m^2 SC D 1–5 + IFNa 2a 5 MIU/ m^2 SC TIW both for 4 wk; 5-FU 250 (350) mg/m^2 IV bolus on D 1–5 [a]
Ravaud et al. *(23)*	111	105	6	100	0	2	2 (0–7)	[a]/34	5FU 600 mg/m^2 CI on D 1–5 Q 4 wk + IFNa-2a 6 MIU SC on D 1,3, 5 QOW for 8 wk IL-2 9 MIU SC QD X 6 d QOW for 8 wk
Ellerhorst et al. *(24)*	55	52	3	[a]	4	12	31 (19–45)	[a]	5FU 600 mg/m^2/d CI on D 1-5 + IL2 2 MIU/ m^2/d CI on D 1-5 + IFNa 4 MIU/ m^2/d SC; one course of therapy = 28 days. [a]
Hofmockel et al. *(25)*	34	34	0	34	3	10	38 (23–56)	[a]/12	IFNa 2 6 MIU/m^2 SC in weeks 1+4, TIW in weeks 2,3; 9 MIU/m^2 TIW in weeks 5-8 + IL 2 20 MIU/m^2 SC TIW in weeks 1+4; 5 MIU/m^2 TIW in weeks 2-3 + 5 FU 750 mg/ m^2 IVb QW in weeks 5-8
Kirchner et al. *(26)*	246	[a]	[a]	[a]	26	54	33[a] (27–39)	[a]	IFNa 2a 6 MIU/m^2 SC on D 1 of W 1+4, D 1,3+5 of W 2–3, 9MIU/m^2 D1,3,5 of W 5-8 + IL 2 10 MIU/m^2 SC BID on D 3,4,5 of W 1+4, 5 MIU/m^2 on D 1, 3,5 of W 2-3 + 5 FU 1000 mg/m^2 IVb QW on W 5-8.
Friedland et al. *(27)*	14	10	4	[a]	1	1	20 (4–56)	1/2	IL-2 6 MIU/m^2/d IV CI X 5 D Q 4-6 weeks + 5 FU 500 (625) mg/m^2/d CI X 5D Q 4-6 weeks + IFNa 2b 5 MIU/m^2 SC Q M,W,F throughout therapy

Ventriglia et al. *(28)*	26	23	3	25	1	7	35 (17–57)	[a]/9	IL-2 10 MIU/m^2 SC on D 1,3,5 of W 1,3,5,7; 5 MIU/m^2 on D 1,3,5 of W 2,4,6,8 + IFNa 2b 5 MIU/m^2 SC on D 2–4 of W 1,3,5,7; 10 MIU/m^2 on D 2–4 of W 2,4,6,8 + 5 FU 400 mg/m^2 IV QW on W 1–8 [a]
Tourani et al. *(29)*	62	60	2	61	1	11	20 (11–33)	[a]/16	IL-2 18 MIU SC TIW + IFNa 9 MIU SC TIW + 5-FU 750 mg IVb QW X 8 wk followed by maintenance Rx.
Fluorouracil+ IFNa									
Gebrosky et al. *(30)*	21	21	0	[a]	4	5	43 (23–66)	[a]/[a]	5-FU 200 (300) mg/m^2/d CI + IFNa 1 MIU SC QD
Lopez et al. *(31)*	33	33	0	25	1	2	9 (2–26)	[a]/2	5-FU 750 mg/m^2 IVb in weeks 1–3 + 5–7 + IFNa 2 10 MIU/m^2 SC TIW X 8 wk
Elias et al. *(32)*	40	40	0	40	0	5	13 (5–28)	[a]/[a]	5-FU 750 mg/m^2/day CI on D 1–5 + IFNa 2b 5 MIU/m^2/d SC on D 1,3,5 Q 21 days
Haarstad et al. *(33)*	31	31	0	30	1	6	23 (10–42)	[a]11	IFNa 12 MIU SC TIW + 5-FU 600 mg/m^2/day CI on D 1–5, then W 3–8: 5 FU 600 mg/m^2 IVb QW + Prednisone 20 mg po QD X 2 wk, then 10 mg po QD. [a]
Igarashi et al. *(34)*	63	53	10	36	3	8	21 (11–35)	[a]/26	Human lymphoblastoid IFN 3 MIU SC TIW + 5-FU 600 mg/m^2/d CI X 5 days on W 1, followed by 600 mg/m^2 IVb QW on W 3–12.
Fotemustine									
Lasset et al. *(35)*	16	14	2	10	0	0	0 (0–27)	[a]/16	Fotemustine 100 mg/m^2 IV on D 1,8,15 followed by a 5-week rest period. Maintenance dose: 100 mg/m^2 IV Q 3 W.
Gemcitabine									
Mertens et al. *(36)*	18	18	0	18	0	1	6 (0–29)	[a]	Gemcitabine 800 (1250) mg/m^2 IV D 1,8,and 15 Q 4 W
Homo-harringtonine									
Witte et al. *(3)*	15	14	1	15	0	0	0 (0–27)	[a]/[a]	4 mg/m^2/d CI X 5 days Q 4 W
Indomethacin + IL-2									
Mertens et al. *(37)*	32	25	7	24	2	3	20 (8–41)	3/[a]	Indomethacin 50 mg (75mg) po Q 8 h (1 W before IL-2) + IL-2 18 *(36)* MIU/m^2/d CI on days 1–5,12–16,+ 23–27

Levamisole+ IL-2									
Creagan et al. *(38)*	25	22	3	22	0	1	5 (0–25)	[a]/28	Levamisole 50mg/m^2 po TID x 5 days + IL-2 3 MIU/m^2/d X 5 days [a]
Linomide									
Pawinski et al. *(39)*	72	63	9	[a]	1	2	5 (1–14)	[a]/9	Linomide 5 mg po BIW (dose escalation: 2nd week-10 mg, then 15 mg thereafter.)
de Wit et al. *(40)*	35	29	6	29	0	0	0 (0-15)	[a]/5	Linomide 5mg po QD X 2 weeks, then 7.5 mg po QD X 2 wk, followed by 10mg QD thereafter.
LY231514-Multi-Targeted Antifolate									
Sauter et al. *(41)*	22	16	6	22	0	1	6 (0–32)	[a]/8	MTA 600 mg/m^2 IV Q 3 W
Megestrol +Interferon Alfa-2b									
Collichio et al. *(42)*	15	15	0	10[a]	0	0	0 (0–25)	[a]	Megestrol 80 mg po BID + IFNa -2b 10MIU/m^2 SC X 5 days QW
Onconase									
Dumas et al. *(43)*	14	[a]	[a]	9	0	0	0 (0–27)	[a]	Onconase 480 mcg/m^2 IV QW
Paclitaxel									
Walpole et al. *(44)*	12	12	0	[a]	0	0	0 (0–30)	[a]/0	Taxol 250 mg/m^2 IV for 24 h Q 3 W
13-*cis*-Retinoic acid									
Berg et al. *(45)*	26	25	1	21	0	0	0 (0-17)	0/8	13-*cis*-retinoic acid 1 mg/kg/d orally
13-*cis*-Retinoic acid +Interferon Alfa									
Elsasser-Beile et al. *(46)*	4	4	0	4	0	0	0 (0–60)	3/13	IFNa 5 MIU/m^2 SC TIW + 13-*cis* retinoic acid 0.3 mg/kg/day X 5 days QW
Motzer et al. *(47)*	44	43	1	37	3	10	30 (18–46)	4/[a]	IFNa -2a 3 (9) MIU SC QD + 13-*cis*-retinoic acid 1 mg/kg/d orally
Retinoid Acid + Interferon Alfa+ Interleukin 2									
Stadler et al. *(48)*	48	47	1	45	0	7	15 (7–29)	1/7	CRA 1 mg/kg/day X 6 W + IFNa 9 or 10 MIU SC BIW X 4 weeks + IL 2 11 MIU SC X 4 days QW X 4 W

All-Trans Retinoic Acid + IFNa									
Escudier et al. *(49)*	31	31	0	31	0	1	3 (0–19)		ATRA 45 mg/m^2/d po QOW + IFNa 18 MIU SC TIW
Tamoxifen									
Shomburg et al. *(50)*	62	59	3	27[a]	0	1	2 (0-10)	[a]/10	Tamoxifen 100 mg/m^2 po QD
Tegafur + Tamoxifen									
Wada et al. *(51)*	10	10	0	7	1	3	40 (14–73)	[a]/2	Tegafur 800 mg/body/ day po + Tamoxifen 20 mg/body/day po
Tegafur + Adriamycin+ Methotrexate+ Tamoxifen									
Wada et al. *(52)*	8	8	0	6	2	2	50 (17–83)	[a]/1	Tegafur 800 (1200) mg po QD + Tamoxifen 20 mg po QD and Adriamycin 20 mg IV + Methotrexate 10 mg IV alternately at two-week intervals.
Temozolomide									
Di Palma et al. *(53)*	12	12	0	12[a]	0	0	0 (0–30)	[a]/1	Temozolomide 200 mg/m^2/day po on D 1–5 Q 4 W
TNP-470									
Stadler et al. *(54)*	33	20	13	[a]	0	1	5 (0–27)	1/3[a]	TNP-470 60 mg/m^2 IV 3 days/week
Topotecan									
Law et al. *(55)*	15	14	1	15	0	0	0 (0–27)	2/5	Topotecan 1.5 (1.75) mg/m^2/day IV X 5 days Q 4 W
Toremifene									
Gershanovich et al. *(56)*	36	36	0	36	1	5	17 (7–34)	[a]/10	Toremifene 300 mg po q day
Vinblastine + Acrivastine									
Berlin et al. *(57)*	17	15	2	17	0	0	0 (0–25)	[a]/3	Vinblastine 1.6 mg/ m^2/day CI X 4 days + Acrivastine 400 mg Q 4 h X 6 D
Vinblastine + Dexverapamil									
Mickisch et al. *(58)*	18	13	5	13	0	1	8 (0–38)	[a]/7	Vinblastine 1.4 (2.0) mg/m^2 CI X 5 D + Dexverapamil 1.5 (3) gm po qd on D 0-6 Q 3 W
Motzer et al. *(59)*	25	23	2	22	0	0	0 (0–18)	[a]/6	Vinblastine 0.11 mg/kg IV on Days 1,2 + Dexverapamil 120 (240) mg/m^2 po Q 6 h X 12 doses Q 3 W

Vinblastine + Dipyridamole									
Murphy et al. *(60)*	15	15	0	15	0	0	0 (0–25)	[a]/2	Vinblastine 0.2 mg/kg IV + Dipyridamole 75 mg po QID (48 h before + continuing 48 h after vinblastine) Q 3 W
Vinblastine + IL2									
Indrova et al. *(61)*	8	7	0	7	0	1	14 (1–58)	[a]/3	Vinblastine 5 mg/m^2 IV QW during weeks 4–5 + IL-2 18 MIU/m^2 SC BID X 5 D on week 1, then 9 MIU/m^2 BID on D 1–2 followed by 18 MIU/m^2 BID on D 3–5 during weeks 2,3,5,6,7.
Tabernero et al. *(62)*	26	23	3	[a]	0	2	9 (2–30)	[a]/9	IL-2 18 MIU/d SC D1-2, then 6 MIU/d D3–5, followed by D1–5 QW X 5 wk + Vinblastine 5 mg/m^2/ d IV QW X 6 weeks followed by maintenance Rx.
Vinblastine + IL2+ IFNa									
Pectasides et al. *(63)*	31	31	0	31	4	8	39 (22–58)	[a]/10	IL-2 4.5 MIU SC Q 12 h TIW X 2 weeks + IFNa 3 MIU SC TIW X 2 weeks + Vinblastine 4 mg/m^2 IV Q 3 weeks
Vinblastine + IFNa									
Lopez et al. *(64)*	20	20	0	[a]	1	2	15 (4–39)	[a]/13	IFNa 12 MIU/m^2 SC TIW + Vinblastine 6 mg/m^2 IVb in W 2,5, and 8.
Kellokumpu-Lehtinen et al. *(65)*	30	30	0	[a]	0	5	17 (6–36)	[a]/[a]	Vinblastine 0.1 mg/kg IV q third week + IFNa 2a IM TIW: Dose 1–2: 3 MIU, 3–4: 9 MIU, Dose 5 to last dose: 18 MIU
Dose									[a]
Papadopoulos et al. *(66)*	50	50	0	[a]	2	13	30 (18–45)	[a]/2	IFNa2b5 *(10)* MIU/ m^2 SC TIW + Vinblastine 0.1 mg/kg IV Q 3 W

Vinblastine + 5-FU + 13-Cis-Retinoic acid + IL-2 + IFNa Azpodien et al. *(67)*	24	24	0	18	4	6	42 (23–63)	[a]/13	IL-2 10 MIU/m^2 SC BID D 3–5 on W 1,4; 5 MIU/m^2 D 1,3, 5 on W 2, 3 + IFNa 6 MIU/m^2 SC D 1 on W 1,4, then D 1,3,5 on W 2,3; 9 MIU/m^2 D 1,3,5 on W 5–8 + 5-FU 1,000 mg/m^2 IV D 1 on W 5–8 + Vinblastine 6 mg/m^2 IV D 1, W 5, 8 + 13-CRA 35 mg/m^2 po QD on W 1–8
Vinblastine + Doxorubicin + IFNa Jekunen et al. *(68)*	11	11	0	11[a]	0	2	18 (3–52)	[a]/3	Vinblastine 4 mg/m^2 IV QW + Doxorubicin 12 mg/m^2 IV QW + IFNa2a 3 MIU SC TIW in week 1, 9 MIU TIW in week 2, then 18 MIU TIW thereafter.

[a] Unclear, not stated, or cannot determine.

Table 2
Randomized Trials

Reference(s)	*Arm*	*No. Entered*	*Response Rate*	*Median Survival (months)*	*Initial (highest) Dose*
Minasian et al. *(71)*	A	25	4%	11.4[a]	IFNα2a 3 MIU/m^2 SC X 3 days, 9MIU/m^2 SC X 3 days, 18 MIU/m^2 SC X 3 days, then 36 MIU/m^2 SC until POD
	B	28	3.6%	10.3 (p = 0.97)	Arm A + vinblastine 0.15 mg/kg IV q 3 weeks
Negrier et al. *(72)*	A	70	1.4%	N/A	IL-2 9 MIU SC on days 1 to 6 of wks 1,3,5,7; IFN 6 MIU SC on days 1,3,5 of wks 1,3,5,7
	B	61	8.2% (p = 0.10)		Arm A + 5-FU 600 mg/m^2/d CI X 5 days on weeks 1+5

N/A-Not available.
[a] All IFNα trials were included (n = 131).

In some instances, SD includes patients who demonstrate an increase >25% or >50%; hence, are included in the nonresponding category. This can significantly alter the percentage of patients categorized as stable. The importance of a uniform system for evaluating and reporting stable disease cannot be overemphasized. Variability in the true rate and duration of stable disease can clearly be influenced by the frequency which a patient is evaluated. Because the category of SD may be particularly germane to chemo-immunotherapy trials, we reported these patients in this review separately, where possible.

Because many studies had complicated schedules and dosing, only the initial dose and the maximum achieved dose were included in Table 1. As reported in most phase II studies reviewed, the overall response rate included complete and partial responses only. Some investigators used the number of evaluable patients to determine the response rates, whereas others used the total number of patients entered in the study. In order to perform a valid comparison of all the trials, the number of evaluable patients was used to determine response rates.

2. PHASE II TRIALS

2.1. Antimitotics and Spindle Inhibitors

This group of drugs have been studied extensively with previous phase II trials reporting a response rate of 5.6% (95% CI at 3.8 to 7.9%) in 554 adequately treated patients *(2)*. Since 1995, several investigators have continued to evaluate antimitotics either as single agents or in combination with agents known to inhibit the function of P-glycoprotein. To evaluate whether inhibition of multidrug resistance enhances response, patients were treated with vinblastine combined with agents such as acrivastine, dexverapamil, as well as dipyridamole. Acrivastine and vinblastine were administered to 17 patients without response *(57)*. Two trials studied vinblastine and dexverapamil (43 cases) with only one partial response *(58,59)*. In 15 patients treated with the combination of Vinblastine and Dipyridamole, no responses were observed *(60)*. Thus, in these 66 adequately treated patients (75 entered), only one partial response was observed, (1.5%; 95% CI at 0.1 to 9.2%) making this line investigation unfruitful for further study.

The combination of vinblastine and IL-2 was evaluated in 34 patients. One partial response was observed for an overall response rate of 14.3% (95% CI at 0.8 to 58%). A second group obtained two partial responses for a response rate of 8.7%. (95% CI at 1.5 to 29.5%) *(61,62)*.

Three trials (100 cases) combined vinblastine with interferon alpha (IFNα). One study (30 cases) produced five partial responses resulting in a response rate of 16.7% (95% CI at 6.3 to 35.5%) *(65)*. Another group (50 cases) obtained two complete responses and 13 partial responses for an overall response rate of 30% (95% CI at 18.3 to 44.8%) *(66)*. A third study observed a response rate of 15% in 20 patients (95% CI at 4.0 to 38.9%) *(64)*.

Vinblastine was also combined with IL-2 and IFNα (31 cases) producing 2 CR and 8 PR for a response rate of 38.7% (95% CI at 22.4 to 57.7%) *(63)*. One trial studied the combination of vinblastine, 5-FU, 13-Cis-Retinoic acid, IL-2, and IFNα (24 cases). There were four CR and six PR resulting in an overall response rate of 41.7% (95% CI at 22.8 to 63.1%) *(67)*. One study evaluated the combination of vinblastine, doxorubicin, and IFNα demonstrating two PR with an overall response rate of 18.2% (95% CI at 3.2 to 52.3%) *(68)*.

Another drug in this category is docetaxel, a taxane with the ability to inhibit mitosis by promoting microtubule assembly and stabilizing microtubules *(69)*. In 32 patients treated, one partial response (3.7%, 95% CI at 0.2 to 20.9%) was observed *(5)*. Twelve patients received the other taxane, paclitaxel, without any response (0%, 95% CI at 0 to 30.1%) *(44)*.

In summary, of 351 patients entered and 332 adequately treated, a response rate of 16.9% was observed (95% CI at 13.1 to 21.5%). This response rate is higher than the one previously reported by Yagoda et al. (5.6%, 95% CI at 3.8 to 7.9%) *(2)*. The higher response rate is most likely because of the addition of immune modulators to these antimitotic regimens, and not to improved efficacy of these cytotoxic agents.

2.2. Hormones

Tamoxifen, toremifene, and flutamide have been examined previously. This group of hormones is known to induce responses in approximately 7% of patients. Flutamide and IFN has also been reviewed and found to induce responses in 26.9% of patients *(2)*.

Since the last review, one trial studied the combination of immunotherapy and hormonal treatment. This study (15 cases) combined megestrol acetate and interferon α-2b producing no responses (0%, 95% CI at 0 to 25.3%) *(42)*. In another trial (36 cases) toremifene produced one CR and five PR for an overall response of 16.7% (95% CI at 7.0 to 33.5%) *(56)*. Tamoxifen was administered to 62 patients producing one partial response for an overall response rate of 1.7% (95% CI at 0.1 to 10.3%) *(50)*. Tamoxifen has also been combined with other chemotherapeutic agents (*see* Subheading 2.3.). Thus, low response rates continue to be observed with hormonal agents.

2.3. Alkylating Agents

In the1994 review, Yagoda et al. examined trials studying different alkylating agents such as nitroso- and sulfonylureas, cyclophosphamide, ifosfamide, and melphalan. A total of 650 patients were entered. The overall response rate was 2.6% (95% CI at 1.5 to 4.2%) for all studies *(2)*.

Since then, fotemustine, a new chloroethylnitrosourea, has been evaluated in 16 patients. There was no response to this treatment (0%, 95% CI at 0 to 26.8%) *(35)*. A new imidazotetrazine derivative, temozolomide, was studied in 12 patients without response (0%, 95% CI at 0 to 30.1%) *(53)*.

2.4. Antimetabolites

In the last review two antifols, trimetrexate and 10-deaza-aminopterin, were evaluated. Of the 60 patients enrolled, only one response was noted for a rate of 1.67% *(2)*.

Methotrexate was evaluated in combination with tegafur, tamoxifen, and doxorubicin (*see* Subheading 2.7.). Edatrexate, a lipophilic methotrexate analogue, was evaluated in 44 patients producing two partial responses for a rate of 5.4% (95% CI at 0.9 to 19.5%) *(11)*. Twenty-two patients received LY 231514, a multitargeted antifolate with the capacity to inhibit several folate-dependent enzymes including thymidylate synthase and dihydrofolate reductase. There was one partial response for a rate of 6.3% (95% CI at 0.3 to 32.3%) *(41)*.

2.5. Pyrimidines

Pyrimidines such as 5-fluorouracil and floxuridine, have been extensively evaluated over the past 18 yr in metastatic RCC. The last review of chemotherapy in RCC found that of 424 patients (466 evaluable) entered in studies evaluating either of these two drugs, a total of 57 patients responded (13.4%; 95% CI at 10.3 to 17.1%) *(2)*. Investigators have continued to publish trials evaluating 5-FU and floxuridine using different schedules, as well as in combination with other agents.

Two trials, treating a total of 59 patients, examined floxuridine administered by continuous infusion. Conroy treated 30 patients with a chronomodulated continuous infusion of floxuridine resulting in four PR for a rate of 14.3% (95% CI at 4.7 to 33.6%) *(13)*. The other trial administered a constant-infusion of floxuridine at 0.075 mg/kg/day over 14 d producing one CR and five PR for a rate of 20.7% (95% CI at 8.7 to 40.3%) *(12)*.

Biological response modifiers such as IFN and IL-2 have been combined with floxuridine. Infusional floxuridine combined with IFNα were examined in two trials totaling 66 patients. Chang found one CR and five PR for a rate of 25% (95% CI at 10.6 to 47.1%) when interferon α-2b was administered at 3.0 MIU/m^2 TIW starting with the second cycle of floxuridine. The dosage of floxuridine was 0.075 mg/kg/d administered over 14 d. *(14)*. Using 10 MIU of interferon TIW and a slightly lower dose of floxuridine, Falcone et al. found three CR and 10 PR for a rate of 33.3% (95% CI at 19.6 to 50.3%) *(15)*.

Fifteen patients received the combination of floxuridine and interleukin-2 resulting in four partial responses for a rate of 26.7% (95% CI at 8.9 to 55.2%) *(14)*. Floxuridine when combined with both biologic response modifiers, IFNα, and IL-2, produced six PR for a rate of 26.1% in 31 patients (95% CI at 11.1 to 48.7%) *(16)*.

Other chemotherapeutic agents have been combined with infusional floxuridine. A modest response rate was observed when 15 patients were treated with the combination of floxuridine and vinblastine. A response rate of 28.6% (95% CI at 9.6 to 58%) was observed at the cost of increased leukopenia and neurotoxicity when compared to floxuridine alone *(17)*.

Combining all floxuridine-based trials, a response rate of 25% was observed in 172 adequately treated (186 entered) patients (95% CI at 18.9 to 32.3%).

The role of 5-fluorouracil in RCC has been examined extensively and 18 trials were reviewed. Twelve trials studied the combination of 5-FU, IL-2, and IFNα, and five trials evaluated 5-FU combined with IFNα. Another trial examined the combination of 5-FU, vinblastine, 13-*cis*-retinoic acid, IL-2, and IFNα.

Of the 12 trials evaluating the combination of 5-FU, IL-2, and IFNα, four used infusional 5-FU (207 patients), whereas the other eight trials examined short infusion 5-FU.

In the infusional 5-FU group, Ravaud et al. observed a response rate of 1.9% (95% CI at 0.3 to 7.4%), whereas Ellerhorst's and Friedland's trials obtained higher responses: 30.8% (95% CI at 19.1 to 45.3%) and 20% (95% CI at 3.5 to 55.8%), respectively *(23,24,27)*. A study by Sella found three CR and six PR for a rate of 47.4% (95% CI at 25.2 to 70.5%).

Five of eight trials examining 5-FU as a bolus infusion, combined with IL-2, administered subcutanously or intravenously, and IFNα found response rates of at least 33% *(18, 20,25,26,28,18)*. The highest reported response rate was observed by Atzpodien. Seventeen responses were noted in 35 patients (48.6%) *(18)*. Other investigators found responses ranging from 0 to 19 *(21,22,29)*.

In summary, the infusional 5-FU trials induced 8 CR and 21 PR in 186 adequately treated patients (207 entered) for a rate of 15.6% (95% CI at 10.9 to 21.8%), whereas the short infusion 5-FU trials produced 45 CR and 136 PR in 572 adequately treated patients (587 entered) for an response rate of 31.6% (95% CI at 27.8 to 35.6%). Overall, the entire group (5-FU+IL-2+IFNα trials) had 758 adequately treated patients (794 entered) and resulted in response rate of 27.7% (95% CI at 24.6 to 31.1%).

The combination of 5-FU and IFNα was also examined. Five trials were reviewed comprising of 188 patients. The combined response rate for these trials was 19.7 % (95% CI=14.3–26.5%). The schedules of 5-FU included both bolus administration and continuous infusion. Two trials administered 5-FU by continuous infusion in addition to intravenous bolus obtaining response rates of 20.8% (95% CI at 11.3 to 34.5%) and 22.6% (95% CI at 10.3 to 41.6%) *(33,34)*. Two other trials examined continuous infusion 5-FU at dosages of 200 mg/m2/d and 750 mg/m2/d resulting in response rates of 42.9% (95% CI at 22.6 to 65.6%) and 12.5% (95% CI at 4.7 to 27.6%), respectively *(30,32)*. One trial evaluated intravenous bolus infusion 5-FU at 750 mg/m^2 weeks 1–3 and 5–7 combined with interferon 2α 10 MIU/m^2 TIW producing a rate of 9.1% (95% CI at 2.4 to 25.5%) *(31)*.

Two small Japanese studies examined the role of tegafur, a prodrug of 5-FU, in RCC. *(70)* Both studies included tegafur as part of a chemoendocrine regimen. One study combined tegafur with tamoxifen inducing responses in 40% of patients *(51)*. The other study examined the combination of tegafur, adriamycin, methotrexate and tamoxifen in eight patients and also demonstrated high antitumor activity inducing two CR and two PR for a rate of 50% *(52)*.

Eighteen patients received gemcitabine resulting in one response for a response rate of 5.6% (95% CI at 0.3 to 29.4%) *(36)*.

In summary, the pyrimidine trials enrolled 1228 patients (1168 adequately treated) resulting in 74 CR and 233 PR for a response rate of 26.3% (95% CI at 23.8 to 28.9%). As reported in the Yagoda review 5-FU and floxuridine appear more active, but combinations with immunomodulators appear to induce higher response rates *(2)*.

2.6. Retinoic Acid

Retinoic acid has been evaluated as a single agent and in combination with immunotherapy. Five trials were reviewed for a total of 153 patients. 13-*cis*-retinoic acid was

evaluated in 26 patients producing no responders *(45)*. The combination of 13-*cis*-retinoic acid and IFNα resulted in 3 CR and 10 PR for a rate of 30% in one study (44 cases), whereas a smaller study (four cases) induced no remissions *(46,47)*. All-trans-retinoic acid and interferon alfa produced one responder for a rate of 3% in 31 patients *(49)*. Forty-eight patients received 13-*cis*-retinoic acid, IL-2, and IFNα resulting in one CR and seven PR for a rate of 17% *(48)*.

2.7. Anthracyclines and Other Intercalating Agents

Nine anthracycline or anthracycline-like agents, including doxorubicin, idarubicin, and mitoxantrone were examined in the Yagoda review. The entire drug class produced a response rate of 2.9% *(2)*.

Lipodox, a liposomal encapsulated doxorubicin, was evaluated in 14 patients. No responses were observed *(6)*. Another form of liposomal doxorubicin, Doxil, was tested in 32 patients producing one response for an overall response rate of 3.1% *(7)*. Doxorubicin was also evaluated in combination with vinblastine, high-dose tamoxifen and quinine. Tamoxifen and quinine were added in an attempt to modulate multidrug resistance. Seventeen patients were treated with the combination, but there were no responders, consistent with other studies using P-glycoprotein modulators *(8)*.

Echinomycin, an intercalating agent, was examined in two studies. One trial (17 cases) produced no responders, whereas the other (49 cases) resulted in one PR for a rate of 2% *(9,10)*. Another DNA intercalator, amonafide- an imide derivative of naphthalic acid, did not induce remissions in one study (17 cases) *(3)*.

One study examined the combination of tegafur, doxorubicin, methotrexate, and tamoxifen obtaining a rate of 50% (*see* Subheading 2.5.) *(52)*.

2.8. Modulating Agents

Numerous trials have attempted to use an agent to modulate drug activity without success *(2)*. Five trials studying various immunomodulators were reviewed.

Levamisole was administered with IL-2 resulting in one partial response for a rate of 4.5% (25 cases) *(38)*. The EORTC conducted two studies with linomide, a quinoline, for a total of 107 patients. The first study (72 cases) resulted in one CR and two PR for a rate of 4% *(39)*. Based on this result, the second study (35 cases) administered a higher cumulative dose, but there were no responders *(40)*. Another study combined IL-2 and indomethacin resulting in two CR and three PR for a response rate of 16% (32 cases) *(37)*. The combination of coumarin and cimetidine produced a remission rate of 18.9% in 37 patients *(4)*.

2.9. Miscellaneous

Topoisomerase I, an enzyme that is essential for DNA replication, is expressed at significant levels in primary RCC specimens and thus target for cytotoxic therapy. Despite this observation, none of 15 patients were treated with topotecan, a toposisomerase I inhibitor, responded to treatment *(55)*. Renal tumors, which are highly vascular, are ideal for the evaluation of antiangiogenisis drugs. TNP-470, an angiogenesis inhibitor, was tested in 33 patients. Only one partial response was obseved for a rate of 5% *(54)*. Caracemide, (N-acetyl-N, O (methylcarbamoyl) hydroxylamine), which inhibits the synthesis of DNA, RNA, and proteins and homoharringtonine, a cephalotaxine alkaloid that blocks DNA, RNA, and protein synthesis by inhibiting chain initiation, were evaluated in 17 and 14 cases, respectively, without response *(3)*.

Table 3
Comparison of 5-FU Containing Regimens

Trials	*Ravaud et al.*	*Tourani et al.*	*Hofmockel et al.*	*Atzpodien et al.*
No. of patients	111	62	34	35
No. Adequate	105	60	34	35
No. Untreated	100 (90%)	61 (98%)	34 (100%)	31(86%)
Total dose per cycle				
IL-2	216 MIU	216 MIU	150 MIU/ m^2	150 MIU/ m^2
IFNα	72 MIU	108 MIU	129 MIU/ m^2	129 MIU/ m^2
5-FU	6000 mg/m^2	3000 mg	2250 mg/m^2	2250 mg/m^2
Duration of cycle	8 wk	8 wk	8 wk	8 wk
Performance Status[a]				
0	41.8%	60%		74.3%
1	42.7%	40%	80%[b]	25.7%
2	15.4%			
Prior nephrectomy	87.4%	89%	82.4%	97%
Response rate	1.8%	19%	38%	48.6%

[a]ECOG scale.
[b]ECOG < or =1.

2.10. Phase III Trials

Two chemoimmunotherapy randomized studies were reviewed (Table 3). Minasian et al. studied both biologic response modifiers, IL-2 and IFNα, with and without vinblastine *(71)*. The study was closed because of low response rates in both arms. The vinblastine arm produced a response rate of 3.6%, whereas the immunotherapy arm resulted in a rate of 4.0%. The difference in median survival was not statistically significant. A French study examined the combination of IL-2 and IFNα with and without fluorouracil *(72)*. The 5-FU arm showed a rate of 8.2%, and the arm without 5-FU produced a rate of 1.4%. This difference was not statistically significant.

3. DISCUSSION

In this review, a total of 2327 patients entered trials, and 93% or 2169 patients were adequately treated. The overall response rate was 18.5%. In Yagoda's review, a total of 4542 patients entered trials, of which 90% or 4093 patients were adequately treated. The overall response rate was 6.0% (95% CI at 5.3 to 6.8%). It was concluded that the pyrimidines, 5-flurouracil, and floxuridine, were the most active group from the extensive list of drugs reviewed. They were termed agents with modest antitumor activity against renal cell cancer *(2)*. Hence, 5-fluorouracil-containing regimens have been studied the most over the past four years.

Compared with the Yagoda review, which found an overall response rate of 6.0% (95% CI at 5.3 to 6.8%), it would appear that improvements in cytotoxic therapy have been achieved. Unfortunately, this increased response rate represents the additive responses of immunotherapy and minimally or marginally active drugs. Floxuridine as well as 5-FU-based regimens, still appear to have more activity. The relative contribution of immunotherapy to cytotoxic therapy response rate is examined in Table 3. The increased response rates appear to correlate with increased dosages of IL-2, and not with increased dosages of 5-FU. The lower response rate observed by Ravaud was confirmed by Negrier in a

randomized trial where a response rate of 8.2% was observed with the same dosages of IL-2, IFNα, and 5-FU *(72)*. Thus, all chemoimmunotherapy trials must be critically analyzed for dose and schedules.

Clearly, new targets and drugs need to be identified, if response and survival are to be improved.

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19 Interleukin-2 in Metastatic Renal Cell Carcinoma

Thomas Olencki and Ronald M. Bukowski

CONTENTS

1. INTRODUCTION

Interleukin-2 (IL-2) was first described as a T-cell growth factor (TCGF) in 1976 by Morgan et al. when it was noted that conditioned medium could support T-cell growth *(1)*. IL-2 was later demonstrated to have no direct antitumor activity, but to mediate antitumor activity indirectly through the host immune response *(2,3)*. The primary source of IL-2 is from an antigen-stimulated TH1-type CD4+ T cell and, to a lesser extent, from

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activated CD8+ cells *(4)*. At least two external signals are required for T-cell activation, one through the T-cell receptor complex (TCR/CD3+), and the second from an accessory cell expressing B7, a ligand for the T-cell CD28+ receptor. It is encoded on chromosome 4q26-28 *(5)* and was first cloned in 1983 *(6)*. IL-2 is a 15 kD protein consisting of two paired α helices bound by an interchain di-sulfide bond between cysteine residues at positions 58 and 105. IL-2 interacts with the T-cell IL-2 Rβγ heterodimer at the receptor, the structure of which is similar to that of IL-4 and GM-CSF *(7)*.

The functional IL-2 receptor is composed of three subunits, IL-2Rα, IL-2Rβ, and IL-2Rγ. The IL-2Rα (CD25$^+$) was the first subunit characterized and has a molecular weight of 55 kD (p55). It is a transmembrane protein with a large extracellular region, the only known function of which is to bind IL-2. It is upregulated by IL-2 and it may be part of a positive feedback loop for T-cell activation. IL-2Rα is a low affinity receptor ($K_d = 10^{-8}$ *M*). It is thought that the IL-2 molecule first binds IL-2Rα, which then facilitates association with the IL-2Rβγc *(8)*. Circulating levels of IL-2Rα have been found in some disease states but have no known functional role. IL-2Rβ (CD 122$^+$) is a 70–75 kD protein (p70–75). This subunit has an intermediate binding affinity ($K_d = 10^{-9}$*M*) for IL-2. Finally, IL-2Rγ has a molecular weight of 64 kD (p64) and forms a heterodimer with IL-2Rβ. The resulting receptor, IL-2Rβγ, has an intermediate binding affinity ($K_d \sim 10^{-9}$ *M*). The complex is critical for IL-2 internalization and induction of the proliferative signal. Both the IL-2Rβ and the IL-2Rγ chains are members of the Type I cytokine receptor family *(9)*. The complete IL-2 receptor, the IL-2Rαβγc trimeric complex, has high affinity binding ($K_d = 10^{-11}$ *M*) for IL-2, thus low levels of exogenous IL-2 can activate T cells. Within 15-30 min of IL-2 binding, the IL-2/IL-2Rαβγ complex undergoes endocytosis and degradation by lysozymes.

IL-2 stimulation induces dimerization of the cytoplasmic domains of IL-2Rβ and IL-2Rγ and aggregation of JAK 1 and JAK 3 that associates with them, respectively. This induces phosphorylation of the JAK kinases and subsequent activation of downstream STAT 5 and STAT 3 *(10)*. The STAT 5 and STAT 3 proteins then migrate to the nucleus were they bind to IL-2 transcription factors.

2. BIOLOGICAL ACTIVITY OF RECOMBINANT IL-2

On May 5, 1992, high-dose IL-2 was approved by the Food and Drug Administration (FDA) for the treatment of metastatic renal cell cancer (RCC). The dose and schedule were based on the reported data from 255 patients treated with high-dose IL-2. Treatment consisted of 600,000 IU/kg IL-2 intraveneously every 8 h for 14 doses over 5 d and was then repeated after a 9-d rest period *(11)*.

The dose of IL-2 is expressed as international units (IU) as defined by the World Health Organization (WHO) *(12)*. As a matter of reference, 6 IU are equivalent to 2 Roche units and to 1 Cetus unit, as described in early publications. A recent publication cautioned that this equivalence has been defined in vitro and may not apply when one attempts to achieve similar biologic effect in vitro *(13)*. The gene was isolated and cloned in 1983 *(6)* and produced using recombinant technology in a genetically modified *Escherichia coli (14)*. The recombinant form differs from the native protein in that it is nonglycosylated, by the lack of the N-terminal alanine and a serine substitution for cysteine at amino acid position 125. As currently produced, 1.1 mg of Chiron IL-2 is equivalent to 18 million ($18 \leftrightarrow 10^6$) IU *(11)*.

3. PHARMACOKINETICS

In the mid-1980s, IL-2 was found to have a $T_{1/2}\alpha$ plasma clearance of 6–10 min and a $T_{1/2}\beta$ extravascular distribution of 30–120 min consistent with a two-compartment model. Greater than 95% of injected IL-2 is cleared within 30 min *(15)*. These data have been confirmed more recently in 1997 by Yang et al. *(16)*. Serum levels are proportionate to the dose of IL-2 administered with 30% of the dose detectable in plasma immediately after infusion *(11)*. IL-2 clearance occurs primarily in the kidney by glomerular filtration and extraction by the proximal tubules. No intact IL-2 is detectable in the urine *(17,18)*. The NCI determined that with a subcutaneous (sc) dose of 250,000 IU/kg, serum levels peaked 2–3 h with a $T_{1/2}$ of 5.3 ± 1.9 h *(16)*. In the group of six patients tested, all had IL-2 levels of 1–2 ng/mL 10 h after injection.

4. PHARMACODYNAMICS

This cytokine has wide-ranging effects, which include: proliferation and increased cytotoxicity of antigen-activated T cells, T-cell cytokine (IFNγ) and protein production (perforin and granzyme B), B-cell proliferation with stimulation of Ig secretion, increased NK-cell proliferation, and cytolytic function and increased macrophage cytotoxicity with TNFα, IL1, IL-6, IL-12, and GM-CSF secretion *(8,19)*. Additionally, chronic high levels of IL-2 enhance expression of *Fas* ligand (CD95) on stimulated T cells, which may result in an increase in *Fas*-mediated T-cell apoptosis *(20)*. IL-2 has also been shown to decrease neutophil chemotaxis, which may increase the infection rate in patients undergoing therapy with high-dose IL-2 *(21)*. Endogenous hormone levels may be altered with increases seen in atrial natriuretic factor (ANF), adrenocorticotropic hormone (ACTH), and β endorphin and decreases in melatonin *(8)*.

5. HIGH-DOSE IL-2 AND LAK CELLS

Shortly after the discovery of IL-2, murine studies with intraperitoneal (ip) administered IL-2 determined that: (1) for a given dose, a divided 3 times/d dose was more effective than a single daily injection *(22)*; (2) that more total IL-2 could be administered by 3 times daily dosing than by continuous iv infusion *(23);* and (3) that the greater the dose the higher the response rate *(24)*. In view of the above, phase I trials with an every-8-h schedule were begun in 1984 *(25)*.

Subsequently, IL-2 based murine studies with and without lymphokine-activated killer (LAK) cells *(26,27)* suggested that the two had synergistic activity when used together, however, responses were seen when high-dose IL-2 was used alone *(28,29)*. In view of the above, in the early 1980s, phase I trials with an every-8-h schedule were initiated with IL-2 alone in 39 patients and with LAK cells alone on a once-daily schedule in 26 patients *(23,30)*. No responses were seen. In 1985, Rosenberg et al. reported the NCI results of high-dose IL-2 with LAK cells in the treatment of patients *(31)*. High-dose IL-2 at 600,000 IU/kg (24 MIU/m^2) was given iv bolus every 8 h for 5 d, followed by a 6-d break and another 5-d IL-2 bolus infusion during which LAK cells were infused. Of three patients with RCC, all had a partial response of their pulmonary disease. This initial report was updated in 1987 *(32)* and then later in 1989 *(33)*. Of 72 RCC patients treated at the NCI with high-dose IL-2 and LAK cells, the overall response rate (RR) was 35% with a 24% PR (17 patients) and an 11% CR rate (8 patients). At the time of publication,

the median response duration of complete responses (CRs) was 14 mo and the partial responses (PRs) was 7 mo. However, in a recent 1997 update, only one of the eight patients has remained in CR *(34)*.

To confirm these findings, a confirmatory study was conducted by the Extramural Interleukin-2/LAK Working Group (ILWG) *(35)*. The dose and schedule of IL-2 and LAK cells used by the NCI remained unchanged. Of 32 patients, there were 2 CRs and 3 PRs for a 16% response rate. The median duration of response for the CRs was 10.5 mo and 15.5 mo for the PRs. A review of patient characteristics revealed a heavier tumor burden with more abdominal disease, masses >100 cm^2 and more intact primaries among patients treated in the ILWG trial as compared to the NCI. It was proposed that these differences in patient characteristics might explain the differences in response rate.

6. CIV IL-2 AND LAK

In view of the short half-life and significant morbidity associated with high-dose bolus IL-2, attempts were made to modulate the toxicity. The first reported effort was by William West et al. *(36)* of the National Biotherapy Study Group. In a phase I/II study, patients with metastatic cancer received continuous intravenous (CIV) IL-2 at 6–30 MIU/m^2/d on days 1–5 and 10–15 with LAK cells infused on the later 5 d of IL-2 infusion. Of six patients with RCC, three achieved a PR. Median response duration was 2 mo. The characteristic toxicities of IL-2 developed in most patients, but the grade was significantly less, with few patients needing intensive care unit support. Although the total dose administered was less than by bolus schedules, the regimen was biologically active, with rebound lymphocytosis noted.

Shortly thereafter a phase I study of CIV IL-2 and LAK was performed by the Extramural ILWG *(37)*. Although not part of the phase I study, it was determined that 18 MIU/m^2/d ↔ 4.5 d could be safely administered.

Subsequently, a hybrid schedule of IL-2 administration was developed by the ILWG to improve activity and determine whether the toxicity of CIV IL-2 was less than that of bolus dosing *(38)*. High-dose bolus IL-2 was administered at 720,000 IU/kg every-8-h days 1–3, followed by IL-2 CIV at 18 MIU/m^2 days 9–15 with LAK infusion. Of 47 patients enrolled, there were 2 CRs and 2 PRs for an overall response rate of 9% despite a more favorable patient profile. Of significance, the toxicity of the CIV IL-2 was found to be much greater than the bolus schedule *(35)*.

As above studies raised the issue of how CIV IL-2/LAK compared to the original regimen of high-dose bolus IL-2/LAK in terms of efficacy and toxicity, the Extramural ILWG conducted a randomized phase II study of the two schedules *(39)*. The CIV dose was 18 MIU/m^2/d ↔ 5 d. The remainder of the doses and schedules were as previously described. Nearly all patients had had a nephrectomy prior to entry on the study. Slightly more patients with hepatic metastasis and prior therapy were on the CIV arm of the study. In absolute numbers, approximately four times the IL-2 was administered in the bolus arm compared to the CIV arm. Grade 3/4 toxicities were very similar, except for an increase of fever and infection in the CIV arm, suggesting equivalent biological dosing. On the CIV arm (48 patients) there were 2 CRs/5 PRs for a 15% response rate and on the bolus arm (46 patients) there were 3 CRs/6 PRs for a 20% response rate. Responses on both arms were durable. Although not statistically powered as a phase III study, the authors concluded that both schedules had equal antitumor efficacy, but with a slight increase in toxicity of the CIV arm.

The National Biotherapy Study Group updated their experience with the original West et al. *(36)* regimen of CIV IL-2 *(40)*. Of 167 RCC patients treated by a variety of protocols, all of which incorporated the previously described 5-d IL-2 infusion, there were 3 CRs and 10 PRs for a 8% response rate with a 9.3-mo median survival. Of note they found no differences in overall survival among the protocol subsets in which CIV IL-2 was combined with LAK, TIL, IFNα, TNF, or cytoxan.

7. HIGH-DOSE IL-2 VS IL-2/LAK

As early murine studies demonstrated activity of high-dose IL-2 alone, and as the generation of LAK cells was cumbersome and expensive, IL-2 as a single agent was piloted. Reporting on the cumulative NCI experience with high-dose IL-2 alone, Rosenberg et al. *(33)* described 54 evaluable RCC patients with 4 CRs and 8 PRs for a 22% response rate. However, as the question regarding the need for LAK cells remained, a prospective randomized trial of high-dose IL-2 with or without LAK cells was begun in patients with metastatic cancer *(41)*. The NCI schedule of IL-2 administration was maintained with +/– infusion of the LAK cells. Of the 97 evaluable patients with RCC, 7 CRs and 8 PRs in the IL-2/LAK arm ($n = 49$) and 4 CRs and 6 PRs in the IL-2 arm ($n = 48$) were noted, for an overall response rate of 30.6% and 20.8%, respectively. This was not statistically significant (no p value given). Overall survival at 48 mo was 29% and 25%, respectively ($p = 0.52$).

A similar trial was conducted by the Modified Group C *(42)*. Among 37 RCC patients treated with high-dose IL-2 there was in 1 CR and 2 PRs (8% response rate) and treatment of 32 RCC patients with IL-2/LAK resulted in 4 PRs (13% response rate). No significant differences were noted in the response rate and median survival.

8. CIV IL-2 VS CIV IL-2/LAK

A somewhat similar phase III study was conducted by Law et al. *(43)*, in which patients were randomized between CIV IL-2 at 9 MIU/m^2/d for 4–5 d four times monthly +/- LAK cells. Thirty-four patients treated with IL-2 had 1 CR and 2 PRs (9% response rate) and 32 patients treated with IL-2 and LAK cells had 1 CR and no PRs (3% response rate) for an overall response rate of 4%. There were no differences in response rate ($p = 0.61$) and survival ($p = 0.47$) between the two arms. With the completion of these three randomized trials, it was concluded that LAK cells were no longer needed in the treatment of patients with metastatic RCC.

9. CIV IL-2

To deliver equivalent biologic doses without the need of an ICU, continuous-infusion IL-2 administration has been used in a wide variety of doses and schedules. It remained the predominant mode of delivery used in Europe until the recent widespread use of SC IL-2. As approved for use in Europe, most of the early trials were conducted with IL-2 at a dose of 18 MIU/m^2/d for 5 d, which was repeated after a 1-wk rest. Despite the variety of doses and schedules (Table 1), no dose/response rate relationship has been observed for continuous infusion IL-2.

10. HIGH-DOSE IL-2 ALONE

In contrast to the results obtained at the NCI, Abrams et al. *(44)* of the ILWG noted no responses in 16 patients treated using the NCI high-dose IL-2 regimen. The lack of

Table 1
Trials of Single Agent rIL-2: Continuous Intravenous Infusion Schedules[a]

Authors	Schedule rILl-2	Dose (MIU)	No. of Patients	CR	PR	Median Response Duration/Median Survival (mo)
Philip et al. *(99)*	Days 1–4	18/m^2/d	60	2	7	8/NS
Palmer et al. *(100)*	Days 1–4	18/m^2/d	92	3	10	12.2/NS
	Days 1–4	18/m^2/d	133	4	11	9.6/NS
Whitehead et al. *(101)*	Days 1–4	13.5/m^2/d	45	0	6	7+/15
Escudier et al. *(102)*	Days 1 and 2	24/m^2/d	40	1	10	NS/15
Lopez et al. *(103)*	Days 1–5, 8–11	18/m^2/d	30	1	3	13/11
Escudier et al. *(104)*	Days 1 and 2	24/m^2/d	104	4	16	13-22+/13
von der Maase et al. *(105)*	Days 1–5, 12–15	18/m^2/d	51	2	6	7, 13.1 (CR); 12.4 (PR)/12.4
Koretz et al. *(106)*	Days 1–4	9/m^2/d	11	0	0	NS
Geertsen et al. *(107)*	Days 1–5, 8–11	18/m^2/d	31	2	4	6.5/8.6
Stoter et al. *(108)*	Days 1–5	3/m^2/d	18	1	2	NS
Law et al. *(43)*	Days 1–5, 8–11, and so on	9/m^2/d	36	1	2	
Negrier et al. *(109)*	Days 1–5, 12–15	18/m^2/d	138	NS	9[b]	NS
Totals (%)			789	21 (2.7)	86 (10.9)	

rIL-2: recombinant human interleukin-2; MIU: million International Units; CR: complete response; PR: partial response; NS: not stated.

Complete response + partial response = 13.5% (95% confidence interval 11.1%-15.8%).

[a] Adapted in part from Bukowski *(112)*.

[b] Total response rate given.

response seen occurred despite favorable patient characteristics, notably a median age of 48, low tumor bulk in 14/16, half with a PS of 0, and 14/16 having had a nephrectomy.

To deliver high-dose IL-2 therapy, but attempt outpatient administration and management, Bukowski et al. *(45)* of the Southwest Oncology Group (SWOG) treated 41 patients with 60 MIU/m^2 intraveneously three times weekly (TIW) until progressive disease or intolerance. Although only three patients required hospitalization, 30 required dose reduction to a median of 50%. One CR and 4 PRs were observed for a 12% response rate. However, eight ineligible patients were included in the calculations for response.

More recently, Oleksowicz and Dutcher *(46)* described a dose-intense regimen of high-dose IL-2 (24 MIU/m^2/dose). Patients with stable or responding disease received up to a total of five cycles of therapy, with each cycle consisting of a maximum of 28 doses of IL-2. Three CRs (15%) and 5 PRs (25%) occurred among the 20 patients treated for a 40% overall response rate. Response appeared to correlate with total dose of IL-2 administered, with a dose intensity of ⊕1440 MIU/m^2/yr significant for predicting complete response.

The marked discrepancy of response noted in these trials of 0–40% suggests that selection factors, magnified by small patient numbers and low response rates, may have a significant role in the outcome of a high-dose IL-2 trial. However, the bulk of trials have more modest response rates (Table 2). An alternative explanation for a dose-intense response rate is that patients who are able to tolerate such therapy are an inherently different population than those whose disease rapidly progresses.

11. HIGH-DOSE IL-2 SUMMARY

Data from the seven trials that represented the database presented to the FDA were summarized by Fyfe et al. *(47)*. The protocols permitted up to 28 doses of IL-2 per course

Table 2
Trials of Single-Agent rIL-2: Bolus Infusion Schedules[a]

Authors	Schedule rILl-2	Dose Range (MIU)	No. of Patients	CR	PR	Median Responses Duration/Median Survival (mo)
Atkins et al. *(64)*	Q 8 h Days 1–5	24/m^2	71	4	8	16+/15.5
Fyfe et al. *(47)*	Q 8 h Days 1–5	0.6–0.72/kg	255	12	24	20.3/16.3
Yang et al. *(55)*	Q 8 h Days 1–5	0.72/kg	65	2	11	NS
	Q 8 h Days 1–5	0.072/kg	60	4	5	NS
Rosenberg et al. *(110)*	Q 8 h Days 1–5	0.72/kg	149	10	20	15/20
Rosenberg et al. *(41)*	Q 8 h Days 1–5	0.72/kg	48	4	6	NS
Taneja et al. *(111)*	Q 8 h Days 1–5	0.6–0.72/kg	28	1	4	NS
Bukowski et al. *(45)*	3 x per wk	60/m^2	41	1	5	5/10.8
Abrams et al. *(44)*	Q 8 h Days 1–5	0.06/kg	16	0	0	NS

rIL-2: recombinant interleukin-2; MIU: million International Units; CR: complete response; PR: partial response; q: every; NS: not stated

Complete response + Partial response = 16.5% (95% confidence interval, 13.8%-19.2%).

[a]Adapted in part from Bukowski *(112)*.

until dose-limiting toxicity, with most patients receiving far less. The trials used IL-2 at 600,000-720,000 IU/kg iv bolus every 8 h, up to 14 doses over 5 d, followed by a 5–9-d rest and then another 5-d course of therapy. Of 255 patients treated with high-dose IL-2 alone, 12 CRs (5%) and 24 PRs (9%) were seen for a 14% response rate (95% CI 10–19%). Responses were durable with a 19-mo median duration for PRs and the median duration had not yet been reached for the CRs. Significant responses were seen in poor prognostic sites, however, the bulk of responses were seen in patients with lung and lymph-node predominant disease. Of note, the only factor predictive of response was performance status. Grade 3/4 toxicity was significant and associated with a 4% mortality. Later reports note a marked decline in mortality with appropriate patient selection.

The responses described above were updated in 1997 *(48)*. There were now 37 patients or 15% who responded with 17 CRs (7%) and 20 PRs (8%). The increase in response rate was in part caused by resection of residual disease in patients with minor and partial responses *(49)*. The median duration of PRs was 20 months and the median duration had not been reached for the CRs.

The dose range of 600,000–720,000 IU/kg often quoted in high-dose IL-2 studies arose from what proved to be a consistent 20% dilutional error (600,000 + 20% = 720,000) that took place in the NCI pharmacy prior to 1993 *(41)*. IL-2 is approved by the FDA for use at a dose of 600,000 IU/kg *(11)*.

12. LOW DOSE IL-2

A number of factors gave rise to the impetus for low-dose IL-2. The significant morbidity associated with high-dose IL-2 required careful patient selection, which dramatically decreased the number of potential patients who might benefit from therapy. Unfortunately, concomitant illness is most frequently found in those who have the highest incidence of RCC. The requirement for careful patient monitoring and occasional medical intensive care have made high-dose IL-2 administration costly and restricted its use to large medical centers. The practical effect of which is to restrict availability to only a minority of patients. Some may also question high-dose therapy given the overall response

rate of 0–15%, with a durable CR rate of 0–4% *(44,47)*. In view of the above, lower doses of IL-2 were critically evaluated.

Using a schedule of CIV infusion, lower doses of IL-2 achieved similar biologic and clinical responses. As SC administration produced prolonged measurable IL-2 levels, attempts were made to use this modality to mimic the therapeutic effect of CIV dosing. Based on preclinical models, earlier efforts at high-dose administration were geared toward the delivery of maximal tolerable doses, whereas lower-dose schedules seek the lowest effective dose necessary to achieve durable response. The correct dose of "low-dose" IL-2 has not been established, but has been described as being at least sufficient to cause a secondary lymphocytosis and eosinophilia. This dose has been found to be at least 1.5 MIU/d subcutaneously, but less than 6 MIU/m^2/d *(8,50)*. A more practical definition proposed by Stadler et al, *(51)* consisted of any dose primarily administered in the outpatient clinic by a practicing medical oncologist.

One of the first published studies of low-dose IL-2 was by Whitehead et al. *(52)*. Patients were given SC IL-2 at 3 MIU/m^2 Monday–Friday for 2 wk, with subsequent dose escalation every 2 wk, as tolerated, to 6, 12, 24, and then 30 MIU/m^2. Maximum achieved doses were 12, 24, and 30 MIU/m^2 in 6, 2, and 2 patients, respectively. The median dose given was 6 MIU/m^2 and median length of therapy was 6 wk. Fourteen patients were evaluable and no responses were seen. Dose-limiting toxicity included decrease in performance status, and increase in fatigue and serum creatinine. With such limited patient numbers, including six patients who had prior therapy, it is difficult to comment on response, however, it is intriguing to see the relatively high doses achieved with this schedule.

Stein et al. *(53)* using very low-dose IL-2 (Bioleukin-Glaxo) noted no lymphocytosis or responses with doses of 0.1–10 mcg subcutaneously daily. However at doses of 100 mcg (0.16 MIU) *(51)*, he noted two PRs in nine patients treated. The small size and short duration of follow-up limit the applicability of this study today, but it does suggest that the doses thought to be necessary for a response in this disease may be lower than previously thought.

One of the largest reports of SC IL-2 administration was by Buter et al. *(54)* in which they presented phase II data on 46 evaluable patients. The first 29 patients were treated with a Monday–Friday 6-wk regimen, but that was later modified to a 4-wk course to ameliorate toxicity. IL-2 was administered at 18 MIU daily the first 5 d, whereas on subsequent weeks the dose was changed to 9 MIU on Monday and Tuesday and 18 MIU Wednesday–Friday. Two patients had a CR (4%) and seven had a PR (15%) for an overall response rate of 20% (95% CI 9–34%). Median response duration was 32 mo for CRs and 8 mo for PRs. Of note, no responses occurred in the 10 patients who had not had a nephrectomy. Few grade 3/4 toxicities were seen and most were mild to moderate consisting of fever, chills, nausea, vomiting, diarrhea, pruritis, and hypotension not requiring pressors. The authors pointed out that the rarity of neuropsychiatric symptoms with this dose and schedule suggests that their appearance should make one think of brain metastases.

The NCI conducted a phase III study in which patients were randomized to high-dose IL-2 (720,000 IU/kg) or low-dose (72,000 IU/kg) both administered every 8 h up to 15 doses over 5 d and repeated again after a 7–10-d rest *(55)*. The lower dose was determined to be the maximally tolerable dose without pressor or ICU support. The cumulative amount of IL-2 administered was 2–4x less in the low-dose arm. Four CR/5 PRs were noted among the 60 patients on the low-dose arm (15% RR) and 2 CR/11 PRs developed among the 65 patients on the high-dose arm (20% RR). At a median of 15 mo of follow-up, their

was no difference in response duration, and at 12 mo no difference in actuarial survival rates ($p = 0.81$). Except for infection, which was higher in the low-dose arm secondary to the lack of prophylactic antibiotics, all other grade III/IV toxicities including thrombocytopenia, malaise, and hypotension were lower in the low-dose arm. Responses were updated in 1997 with a median 52 mo follow-up and 5 CR/6 PRs (10% RR) developed on the low-dose arm with 9 CR/13 PRs (19% RR) on the high-dose arm. The greater response rate in the high-dose arm was of borderline significance ($p = 0.059$) but there remained no difference in overall duration of response and overall survival. This was the first prospective randomized trial of IL-2 dose effect in RCC therapy and the first to question the absolute requirement for high-dose IL-2 therapy to achieve durability of response.

Subsequently, a three-arm prospective randomized study was conducted at the NCI by Yang et al. *(16)*, to determine the durable response rate of SC regimens. Patients were randomized to one of the two previously described treatments or, IL-2 at 250,000 IU/kg/d subcutaneously for the first 5 d of the first week followed by 125,000 IU/kg/d subcutaneously 5 d weekly for the next 5 wk for a 6-wk course. Only patients with sufficient reserve and performance status to be treated by high-dose IL-2 were eligible. Findings are very preliminary, but suggest the lower dose of the SC arm results in responses comparable to that seen with high-dose IL-2.

The study by Yang et al. is significant in that it corrects for differences in performance status that may develop in the process of conducting phase II studies. Patients of lower or borderline status may be entered onto a low-dose IL-2 study, whereas that same patient may not be "eligible" for high-dose therapy. This may have the effect of unfavorably skewing the response duration.

13. HIGH-DOSE IL-2 AND TIL

In 1986, tumor-infiltrating lymphocytes (TILs) were described *(56)*. Murine studies demonstrated TILs to be HLA I restricted and 50–100 times more potent than LAK cells in mediating tumor regression. This led to the first studies of TILs and IL-2 in patients with RCC *(57)*. Although a number of phase II studies did demonstrate responses, patient numbers were small and it was difficult to establish particular trends as to IL-2 dose, schedule, and TIL subsets (e.g., $CD\,4^+$ vs $CD8^+$). In 1994, a large multicenter randomized double-blind study of 96-h CIV IL-2 at 5 MIU/m^2/d +/– $CD8^+$ selected TILs was initiated *(58)*. Of 81 patients randomized to receive TIL/IL-2, 33 did not receive TIL secondary to technical factors and nine did not receive CIV IL-2 because of surgical complications or ineligibility issues. Of 79 patients randomized to placebo infusion/IL-2, 11 did not receive therapy because of surgical complications or ineligibility issues. Thus, on an intent to treat analysis, the TIL/IL-2 and placebo/IL-2 response rates were 9.9%/11.4% and 12-mo survival were 55% and 47%, respectively. The authors conclude that $CD\,8^+$-selected TIL does not improve response or survival in patients treated with CIV IL-2.

14. IL-2 AND IFNα

Preclinical work studying the activity of IL-2 demonstrated a correlation of tumor cell surface class I MHC antigens and the anticancer response *(59)*. Of the cytokines tested, interferon alpha (IFNα) was found to upregulate tumor class I MHC Ag, an effect that suggested synergism with IL-2 *(60,61)*. Synergy was demonstrated when subtherapeutic doses of IFNα and IL-2 when administered separately to murine tumors with no response,

were found to have a 66–90% reduction in tumor size when given together *(62)*. A clinical phase I study with IL-2 and IFNα suggested the combination demonstrated synergistic antitumor activity *(63)*. Of the 175 patients, 62 had RCC with an overall response rate of 34%. This high response rate led to the development of numerous phase II studies with the combination. However, three randomized studies suggest the relative merits of the combination relative to IL-2 alone has yet to be determined.

The Extramural ILWG performed a randomized phase II study of high-dose IL-2 (24 MIU/m^2) vs high-dose IL-2 (14.4 MIU/m^2) and IFNα (IFNα 3 MU/m^2) which were administered every 8 h, days 1–5 and 15–19 *(64)*. The majority of patients in both groups had a prior nephrectomy and were PS 0. Of 71 patients treated with high-dose IL-2, there were 4 CRs and 8 PRs for a response rate of 17% (95% CI, 9-28%). No CRs, but 3 PRs, were noted among the 28 patients treated with the combination, for a response rate of 11% (95% CI, 2-28%). Although response duration was markedly better for IL-2 treated patients, median survival for the IL-2 and IL-2/IFNα arms was 15.5 and 16 mo, respectively. This study demonstrated a response rate consistent with other high-dose trials, but pointed out that most of the responses occurred in patients with low-bulk disease and ECOG performance status of 0. Because of the study design, direct comparisons could not be drawn. However, the lack of statistical difference in response rates and median overall survival suggests that the addition of interferon might not contribute to the antitumor response.

Lissoni et al. *(1)* randomized patients to receive SC IL-2 at 9 MIU every 12 h for 2 d followed by 3 MIU twice daily for 5 consecutive days per week for 6 wk, or the same IL-2 dose with IFNα at 5 MU/m 2 TIW. The trial accepted patients with a 40% performance status or greater. Of 30 patients, 15 per arm, there were no CRs. In the IL-2 arm, 5 PRs (33% response rate) and in the combination arm 4 PRs (27% response rate) were noted. No statistical differences in response rate, duration or overall survival developed between the two arms. However, hematologic and hepatic toxicity was markedly greater in the combination arm. The authors concluded that IFNα does not add to the effectiveness of IL-2, but does increase toxicity.

The Groupe Francais D'Immunotherapie sought to clarify the possible benefits of combined IL-2 and IFNα *(66)*. A total of 414 patients were randomized to therapy with IL-2 (18 MIU/m^2/d ↔ 5 by CIV), IFNα (18 MU subcutaneously TIW) or the combination (with IFNα reduced to 6 MU subcutaneously TIW). Again, most patients had a prior nephrectomy and were PS 0. At week 10, the response rates were 6.5%, 7.5%, and 18.6%, respectively. However, by the week 25 evaluation, response rates had dropped to 2.9%, 6.1%, and 13.6%, respectively. Whereas the improved response of the combined arm remained statistically significant, overall survival at 1 yr was similar at 12, 13, and 17 mo ($p = 0.55$), respectively. The toxicity of the combined arm was similar to that of the IL-2-only arm, but significantly greater than the IFNα arm. The lower response rate of 6.5% seen in the IL-2-only arm is among the lowest values published. This may reflect on the fact that this was a multicenter study, which draws upon patients seen in a routine daily practice and that patients had to have had evidence of clearly progressive disease to be entered. This study is significant in that it represents the largest randomized database of cytokine therapy in this disease.

15. IL-2 AND SEQUENTIAL THERAPY

Although routinely practiced, initiating one biologic therapy after treatment failure with another has failed has rarely been evaluated in a prospective randomized fashion.

Two studies that have looked at this issue have yielded different conclusions. Lissoni et al. *(67)* conducted a phase II study in which patients who progressed on a regimen of IFNα given intramuscularly 18 MU TIW and vinblastine 0.1 mg/kg every 21 d were subsequently treated with IL-2 MIU/ m^2 every 12 h for 2 d followed by 1.8 MIU/m^2 twice daily for 5 d weekly for 6 wk. Four PRs were seen among 13 patients for a 31% response rate. Median duration of response was 9+ mo. One of the responding patients had liver metastasis. The authors concluded that because their data were comparable to that achieved with the combination of IL-2 and interferon, that randomized studies were needed to determine whether interferon added to the results obtained with IL-2 alone.

As part of their multicenter prospective randomized (CRECY) study of IL-2, IFNα, or the combination, Escudier et al. *(68)* permitted patients who failed to respond to single-modality therapy to cross over to the other cytokine. Progression or no response after first line therapy was noted in 48 and 65 patients who received IFNα and IL-2, respectively as previously described *(66)*. Of the 113 patients, there were no CRs and 4 PRs, of which 3 (4.8%) and 1 (2%) were in patients who received IL-2 and IFNα, respectively. All four patients had had a prior nephrectomy, had lung-predominant disease and had a performance status of ECOG 0. There were no differences between the two groups with a median survival of 18 mo for the IFNα and 19 mo for the IL-2 responders. It was concluded that second-line cytokine therapy could at best benefit a minority of high-performance patients, but that survival may be increased in this subgroup. Further studies are warranted.

16. INHALED IL-2

A novel mode of IL-2 administration has been described by Huland et al. *(69)*. Patients with pulmonary predominant disease were administered natural, glycosylated recombinant, or nonglycosylated-recombinant IL-2 four to five times daily by nebulization. Dose intensity of the three cytokines cannot be evaluated as no comparison ratios have been developed. Whereas complete and partial responses were documented, overall numbers were small and therapy mixed, with some patients also receiving systemic IL-2 and or IFNα. Aerosol therapy needed to be daily and continuous to maintain a response. Although there maybe an indication for nebulized IL-2 administration, randomized studies will need to be performed to establish its role in the therapy of metastatic RCC.

17. SPECIAL SITUATIONS

Attempts at retreatment with IL-2 after progression on an IL-2 regimen have rarely been successful. This has been evaluated in a deliberate manner by Sherry et al. *(70)*. Thirty-three RCC patients who relapsed after attaining a CR/PR to IL-2-based therapy were identified. Twenty-two patients were retreated with the identical regimen to which they first responded. Only one patient had a second response which was of 6 mo duration. The conclusion was that after progression, retreatment should be with a regimen different than the one used initially.

Anephric patients with otherwise good performance status maybe considered for SC IL-2 therapy *(71,72)*. Careful evaluation of an anephric patient treated on IL-2, IFNα, and 5-FU regimen *(73)* revealed a fluctuation pattern of IL-2 levels comparable to that seen in patients with normal renal function *(74)*. Serum IL-2 levels trended higher in the anephric patient, however, clinical toxicity was no greater. The similar pattern of IL-2 levels between normal patients and the anephric patient on dialysis suggests a mechanism of clearance different than that of the renal tubules alone. Alternatively, the mecha-

nism of clearance maybe different for SC dosing, as serum levels are 2% and 20% of those found after iv bolus and CIV dosing, respectively. Clearly further clinical study in this area is warranted.

18. TOXICITY

The toxicity of high-dose IL-2 is time- and dose-dependent and reversible after discontinuation of therapy. Toxicity is delayed for several hours after the onset of IL-2 infusion and coincides with the secondary release of cytokines IL-1 *(75)*, IL-6, IL-12, TNF, IFNγ, and GM-CSF *(76–78)*. These cytokines may cause direct endothelial injury, or they may induce production of nitric oxide (·*N* = O) *(79,80)*. The net effect of which is to disrupt the vascular endothelium, permitting extravasation of intravascular fluid to the extravascular/interstitial space. This results in organ edema and with IL-2 induced lymphocytic infiltration, can lead to impaired organ function. The fluid shifts and alteration in organ function are referred to as the capillary leak syndrome (CLS) or vascular leak syndrome (VLS). The constellation of events described is responsible for the many of the toxicities of IL-2. All patients develop constitutional symptoms of fever, chills, and fatigue, the first two of which are readily treated. With time the fever and chills tend to diminish with fatigue remaining prominent.

Significant circulatory and cardiac changes occur that resemble those seen in septic shock. Marked hypotension develops as a result of the loss of fluid from the intra- to the extravascular space secondary to capillary leak and decreased vascular resistance, which also results in reflex tachycardia and occasional atrial arrythmias *(81)*. Lower dose dopamine (5–10 mcg/kg) or phenylephrine are used for pressure support as colloids can percipitate ARDS caused by the capillary leak syndrome *(82)*. Phenylephrine is used if dopamine is not effective because its primary α adrenergic effects do not result in tachyarrhythmias. Myocarditis, pericarditis, and pericardial effusions have been documented, but are less common. Life-threatening arrythmias and myocardial infarction were seen prior to the initiation of diligent cardiac screening.

Aside from hypotension, one of the most dose-limiting toxicities of high-dose IL-2 is seen in the lung. A capillary leak develops at normal pulmonary wedge pressures and manifests as dyspnea at rest with an adult respiratory distress syndrome (ARDS) appearance on radiologic exam *(81)*. The concern of developing progressive pulmonary toxicity prevents aggressive hydration for hypotension. Historically, the ARDS like pulmonary changes were worsened by the infusion of LAK cells. Hypoalbuminemia, infections, and cardiac dysfunction can potentiate the ARDS even after the discontinuation of the IL-2. Less common is the occurrence of pleural effusions.

Nonoliguric renal insufficiency develops in the majority of patients. A combination of decreased renal perfusion secondary to the hypotension and direct proximal tubular damage contribute to the weight gain and edema *(83)*. Diuretics are used primarily in the post-IL-2 period to assist with the excretion of fluid mobilized from interstitial spaces. Renal function returns to normal at the completion of the IL-2 administration.

Most patients develop acalculus cholestatic jaundice with associated rises in alkaline phosphatase *(84)*. Transaminases often remain normal. Marked declines in serum albumin are attributed to extravasation to the extravascular space *(85)*. Hepatic dysfunction and secondary cytokines lead to nausea, vomiting, diarrhea, and anorexia. Gastritis can be seen in all patients and must be treated prophylactically.

Neuropsychiatric side effects including impaired attention and memory, irritability, disorientation, somnolence, encephalopathy, and coma can be seen among patients even with no prior history of such symptoms. Symptoms can worsen/develop after the completion of IL-2 therapy, which requires the discontinuation of therapy at their onset.

Cutaneous changes involve a diffuse erythroderma that may or not desquamate *(86)*. This may become puritic. As with other autoimmune diseases severe cases of psoriasis may worsen. In extreme case, pemphigus may develop *(87)*.

Increases in ACTH, cortisol, cortisol-releasing hormone, epinephrine, prolactin, growth hormone, and β endorphin are seen with IL-2 administration *(88)*. Hypothyroidism has been noted and has both been reported to correlate *(89)* and not correlate *(88)* with response. Others have commented that responders tend to receive and be exposed to a greater number of IL-2 courses and therefore were more susceptible to hypothyroidism *(90)*.

Nearly all patients become anemic and mildly granulocytopenic, which may be because of a decrease in progenitors BFU-E *(91)* and CFU-GM *(92)*, respectively. Thrombocytopenia is noted in most patients but becomes severe <25,000 K/μL in only a minority. Eosinophilia with an associated lymphocytopenia of varying levels is found in most patients.

Early studies with IL-2 revealed a moderate number of septic episodes, many of which were traced to line infections. Among the mechanisms noted, IL-2 inhibition of neutophil chemotaxis was found in patients receiving therapy *(21)*. IL-2 may also decrease the idiotypic response to antigenic stimuli and may reduce the onset of that response by several weeks *(93)*. Antibiotic prophylaxis has reduced infection in patients with indwelling central lines *(94–96)*.

Contrast reactions to ionic and nonionic intravenous contrast dye may happen during or several mo after, the completion of both low- and high-dose IL-2 treatment. Often referred to as a "recall" reaction it includes fever, chills, urticaria, dyspnea, erythematous rash, weakness, and occasionally nausea, diarrhea, and hypotension *(97,98)*. No patient characteristics have been found that predict for the reaction. Premedication with H1 and H2 antihistamines and steroids has proven to be very effective at prevention.

With chronic low-dose therapy, fatigue and anorexia, with subsequent dehydration, replace the acute toxicity of capillary leak syndrome and are the rate-limiting toxicities associated with the therapy. Many of the toxicities described above can occur in low-dose therapy, but to a more moderate degree. Peculiar to SC administered therapy; erythematous raised SC nodules frequently develop at the site of SC injections.

19. CONCLUSIONS

Conclusions that can be drawn based on the available data include:

1. LAK and TIL cells are not needed for and do not improve response to IL-2.
2. Performance status and limited bulk disease in non-direct sites are the two most important factors predictive of response.
3. Although it has been claimed that high-dose bolus IL-2 is associated with durable CRs, this has yet to be adequately addressed in prospective randomized trials of sufficient power and duration to answer this question.
4. The appropriate dose of IL-2 has yet to be determined in prospective randomized trials that overcome imbalances in performance status frequently seen in phase II studies. The NCI sponsored three-arm study as outlined by Yang et al. will begin to address this issue.

5. IFN-α, when used in combination with IL-2, may increase response rates, but no effect on median survival has been demonstrated.
6. A small, but consistent, subset of patients will have prolonged survival after therapy with IL-2.

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20 Role of Interferon in Metastatic Renal Cell Carcinoma

Robert J. Motzer and William J. Berg

Contents

1. INTRODUCTION

The outlook for patients with metastatic renal cell carcinoma (RCC) is poor, with a 5-yr survival proportion of less than 10% for patients presenting with stage IV disease *(1)*. RCC is highly resistant to chemotherapy, with no single agent showing significant antitumor activity *(2)*. Late relapses after nephrectomy, prolonged stable disease in the absence of systemic therapy *(3)*, and rare spontaneous regressions *(4)* were clinical observations that suggested host immune mechanisms could be important in regulating tumor growth and fostered the study of immunotherapy against RCC.

2. TYPES AND EFFECTS

Interferons are members of a superfamily of regulatory proteins called cytokines. These proteins are produced by eukaryotic cells in response to viral infections and to different types of biologic or synthetic inducers, and have complex biological and pharmacological properties. [For review, *see (5)*.] First described in 1957, the name was taken from their ability to mediate viral interference, where one virus interferes with replication of a second *(6)*. In addition to antiviral activity, interferons modulate immune response

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and cell proliferation. Many cells have the capacity to produce these proteins, which may function as local paracrine and autocrine factors in influencing cell growth and function.

More than 20 homologous proteins have been identified. These are divided into distinct antigen subtypes according to differences in antigenic, biologic, and chemical properties. Three subtypes (-α, -β, -γ) were described in humans, had DNA sequenced and cloned, and are commercially available. Interferon-α (IFNα) and interferon-β (IFNβ) are encoded on chromosome 9, share homology, and are collectively referred to as Type I. Interferon-γ (IFNγ) is encoded on chromosome 12, is dissimilar to the other two in structure, binds to a distinct cell membrane receptor, and is referred to as Type II.

Lymphocytes and macrophages produce IFNα in response to viruses, double-stranded RNA, or other inducers. IFNβ is produced by fibroblasts and mesenchymal cells in response to similar inducers as IFNα. IFNγ is produced by T lymphocytes and, to a lesser extent, natural killer (NK) cells. IFNγ is induced during activation of lymphocytes, and has greater immunomodulatory but less antiviral properties than the Type I interferons.

Potential mechanisms for antitumor effect include enhanced tumor immunogenicity, decreased virulence, inhibition of angiogenesis, and inducement of immune response. Activities on the immune system include stimulation of cytotoxic T-lymphocytes (CTLs) capable of recognizing and lysing foreign cells based on MHC class I antigens, upregulation of MHC antigens, and augmentation of antibody-dependent cellular cytotoxicity. Interferons act as positive and negative regulators of NK cells and modify susceptibility of target cells to lysis. IFNγ, in particular, is a potent regulator of macrophage activity.

Interferons have direct effects on tumor cells and surrounding tissues. Antiproliferative effect has been correlated to degree of downregulation of the receptor following interaction with the ligand in vitro *(7)* and in vivo *(8)*. The inhibitory effect is not specific to one phase of the cell cycle, but cells in G_O have been observed to be the most sensitive *(5)*. Antiproliferative effects on endothelial cells and fibroblasts contribute to angiogenic properties. Inhibitory properties on angiogenesis were demonstrated by the successful treatment of hemangiomas with IFNα *(9)*.

Interferons also affect cellular differentiation as either inhibition or enhancement *(10)*. The precise mechanism has not been defined, but this property contributes to the therapeutic efficacy of IFNα against hairy cell leukemia *(11)*. Interferon genes and interferon-induced genes have been shown in vitro to demonstrate tumor suppressor properties, and are considered a class of tumor suppressor genes *(12)*. In summary, interferons have a broad range of effects that could potentially induce clinical responses in patients with RCC. The precise mechanism(s) responsible for clinical response against RCC has not been defined.

3. RESPONSE TO MONOTHERAPY

Antitumor activity against RCC was initially reported in 1983, using a partially purified human IFNα preparation *(13,14)*. Early studies used this preparation *(15)*, or a partially purified lymphoblastoid IFN *(16)*, and reported a response proportion in the 10–20% range. Recombinant technologies resulted in development of highly purified preparations.

Two preparations, recombinant IFNα-2a (Roferon®, Hoffman-LaRoche, Nutley, NJ), and recombinant IFNα-2b (Intron®, Schering-Plough Laboratories, Kenilworth, NJ) are commercially available and represent standard preparations used in clinical practice. Both have been studied extensively against metastatic RCC, with the dose and schedule varied

Table 1
Results with Recombinant IFNα

Source	*Patients Evaluable*	*Dose (↔10^6 IU)/Schedule*	*IFN type*	*No. of CR*	*No. of PR*	*Percent CR + PR*
Minassian *(17)*	39	50 3↔ weekly IM	2a	0	7	18%
Minassian *(17)*	59	3 to 36 QD IM	2a	2	5	11%
Quesada *(18)*	41	20/m^2 daily IM	2a	1	11	29%
	15	2/m^2 daily IM	2a	0	0	0
Umeda *(19)*	108	3 to 36/m^2 QDIM	2a	2	13	14%
Schnall *(20)*	22	3 to 36 QDIM	2a	0	1	5%
Kempf *(21)*	10	2/m^2 3↔ weekly SC	2a	0	0	0
Kempf *(21)*	10	30/m^2 3↔ weekly IV	2a	0	1	10%
Fossa *(22)*	17	18 to 36/m^2 3↔ weekly IM	2a	0	2	11%
Foon *(23)*	21	2/m^2 SQ 3↔ weekly	2b	0	1	5%
Steineck *(24)*	30	10 to 20/m^2 3↔ weekly IM	2a	1	1	6%
Marshall *(25)*	17	1 QD SC	2a	0	4	24%
Umeda *(19)*	45	3 to 36 QD IM	2b	1	7	18%
Muss *(26)*	46	30 to 50/m^2 5↔ weekly q3 weeks IV	2b	1	2	7%
Muss *(26)*	51	2-10/m^2 3↔ weekly SC	2b	1	4	10%
Levens *(27)*	15	10 QD SC	2b	1	3	27%
Bono *(28)*	61	3/m^2 3↔ weekly SC	2b	2	3	8%
Buzaid *(92)*	22	3 to 36 IM QD	2a	0	5	23%
Figlin *(92)*	19	3 to 36 IM QD 5 d/wk	2a	1	4	26%

CR = complete response; PR = partial response.

according to individual trial, from 3 MU to 50 MU/d (Table 1). No difference in efficacy is recognized between the two preparations. Response proportions range between 0 and 30%, and the overall response proportion is 14.5% (13 complete and 81 partial response, 95% C.I. 12–17%) in 648 patients *(17–28)*.

Efforts to define optimal dose have been pursued through randomized trials and retrospective analyses. One randomized trial compared treatment with 2 MU/m^2 to 20 MU/m^2 *(18)*. None of 15 patients responded to the lower dose and this arm of the trial was closed *(18)*. A second trial compared two escalated to 10 MU/m^2 SC versus 30 MU/m^2 IV and showed no difference in response *(26)*. However, the higher dose resulted in excessive toxicity and frequent dose attenuation *(17)*.

The dose of interferon has been divided into three categories: low, medium, and high, corresponding to doses of less than 5 MU/d, 5–20 MU/d, and greater than 20 MU/d, respectively *(29)*. Two retrospective analyses compared dose categorized according to these criteria with the overall response proportion for the published literature. One *(30)* reported higher response proportions in patients treated at the medium dose (5–20 MU/d) and the second *(31)* found the highest response proportions between 5 and 10 MU/d. Because toxicity is dose dependent, a dose of between 5 and 10 MU 3–5 d weekly could be considered optimal.

The average period from start of treatment to an objective response is 3–4 mo *(30)*. Most responses are evident following 2 mo of therapy. The response can be characterized by a slow regression of tumor masses, with patients meeting criteria for a partial response following as long as 12 mo of therapy. The median duration is approximately six months

Table 2
Results with Recombinant Human IFNβ Treatment for Renal Cell Carcinoma

Source	*Patients Evaluable*	*No. of CR*	*No. of PR*	*Percent CR + PR*
Rinehart *(32)*	15	0	2	13%
Kish *(33)*	16	0	1	6%
Nelson *(34)*	15	0	0	0
Kinney *(35)*	25	1	4	20%

PR=partial response; CR=complete response.

Table 3
Results with IFNγ Treatment for Renal Cell Carcinoma

Source	*No. of Patients Evaluable*	*Dose (↔10^6 IU) and Schedule*	*No. of CR*	*No. of PR*	*Percent CR + PR*
Quesada *(36)*	14	5 to 20/m^2 IM	0	1	7%
Quesada *(36)*	16	0.2 to 1/m^2 IV	0	1	6%
Koiso *(37)*	32	8 to 12/m^2 QD IV or IM	0	2	6%
Koiso *(37)*	39	40IV	1	5	20%
Takaku *(38)*	32	8-12/m^2 IV or IM QD	0	2	6%
Takaku *(38)*	30	40 /m^2 IV QD ↔ 5, Q2 wk	1	5	20%
Rinehart *(39)*	13	0.01–75/m^2 IV twice weekly	0	0	0
Garnick *(40)*	41	0.2 to 60/m^2 IV QD	1	3	10%
Kuebler *(41)*	27	0.25/m^2 CIV QD Q4 wk	0	0	0
Aulitzky *(42)*	20	2 SC tiw, Q 2 wk	2	4	30%
Grups *(43)*	9	5 IM QD ↔8, Q 4 wk	0	3	33%
Bruntsch *(44)*	40	2/m^2 IV tiw, Q2 wk	0	1	2%
Foon *(45)*	21	1/m2 SC 3↔/wk	0	1	5%
Ellerhorst *(46)*	34	100 μg SC weekly	1	3	15%
Small *(47)*	202	60 μg/m^2 SC weekly	3	3	3%

and rarely exceeds 2 yr *(31)*. However, long-term survivors following treatment with IFNα were reported *(17)*.

IFNβ and IFNγ have been evaluated in Phase II trials (Tables 2 and 3). The overall response proportion to IFNβ was 11% in four trials comprised of 71 patients *(32–35)*. IFNγ was more extensively investigated, with response proportion range of 0 to 30% in 15 trials of 570 patients *(36–47)*. Interest in IFNγ generated by two reports showing response proportions of 15 and 30% range *(42,46)* resulted in the conduct of a large multicenter Phase II trial *(47)*. Eligibility for this trial required nephrectomy or tumor embolization. The response proportion in 202 patients was 3% *(47)*.

4. EFFECT ON SURVIVAL

A randomized trial compared IFNγ to placebo in 197 patients with advanced RCC *(48)*. The response proportion to placebo was 7% compared to 4% in the group treated with IFNγ *(48)*. There was no significant difference in median time to progression (1.9 mo for both groups) or in median survival (12 mo with IFNγ, 16 mo with placebo) *(48)*. The results of this study *(48)*, and the low response proportion in the multicenter Phase II trial of 3% in 202 patients *(47)*, indicate that IFNγ has no role in single-agent therapy against RCC.

Table 4
Randomized Trials Evaluating Effect of IFNα on Survival in Patients with Metastatic RCC

Author (Reference)	*Treatment*	*No. Patients*	*% Response*	*Median Survival*	*Survival Benefit (p value)*
Steineck *(24)*	Interferon	30	6%	7 mo	No (p = not given)
	Versus				
	Medroxyprogesterone	30	3%	7 mo	
Kriegmar *(49)*	Interferon plus vinblastine	41	35%	16 mo	No ($p = 0.19$)
	Versus				
	Medroxyprogesterone	35	0	10 mo	
Pyrhonen *(51)*	Interferon plus vinblastine	79	16%	17 mo	Yes ($p = 0.0049$)
	Versus				
	Vinblastine	81	2%	10 mo	
Ritchie *(50)*	Interferon	167	16%	8.5 mo	Yes ($p = 0.011$)
	Versus				
	Medroxyprogesterone	168	2%	6 mo	

Four randomized trials addressed the potential role of IFNα in prolonging survival by comparison to treatment with medroxyprogesterone or vinblastine (Table 4). The first two trials *(24,49)* failed to show a benefit, but both were comprised of a small number of patients, and one *(24)* contained a crossover to interferon for the other treatment arm. The two larger, more recent randomized trials reported a small but significant ($p < 0.05$) improvement in survival with IFNα therapy *(50,51)*. In one, IFNα was compared to medroxyprogesterone and resulted in improvement in median survival of 3 mo *(50)*. In the second trial, IFNα plus vinblastine was compared to vinblastine alone, and the combination showed a benefit in median survival of 6 mo for IFNα therapy *(51)*. The addition of vinblastine to IFNα has not been shown to improve survival compared to IFNα alone *(17,52,53)* and several recent trials of vinblastine have failed to demonstrate single-agent activity in RCC *(54–60)*. Therefore, the improvement in survival can be attributed to treatment with IFNα. Whereas these two studies suggested a survival benefit, IFNα therapy results in a low-response proportion and rarity of long-term survival. Moreover, the impact of interferon on quality of life needs to be investigated.

5. PROGNOSTIC FACTORS

Longer survival following treatment with IFNα has been associated with high-performance status, prior nephrectomy, long disease-free interval between initial diagnosis and relapse, and lung-predominant metastases *(17,61)*. A major response proportion (complete plus partial response) of as high as 30% has been reported for patients with prior nephrectomy and lung-only metastasis *(52)*. However, others have found pretreatment features to be less predictive for response, and suggest a 2-mo trial of therapy to identify patients for prolonged therapy based on degree of tumor reduction *(62)*.

The relationship between pretreatment clinical features and survival was studied in 670 patients with advanced RCC treated in 24 Memorial Sloan-Kettering Cancer Center clinical trials of immunotherapy and chemotherapy between 1975 and 1996 *(63)*. This included 328 patients treated with IFNα *(63)*. The median overall survival time was 10 mo *(63)*. Fifty-seven (8%) of 670 patients remained alive and the median follow-up time for the survivors was 33 mo. The proportion of patients surviving at 1 yr was 42%; the 2- and

3-yr survival proportions were 20 and 11%, respectively. Survival was greater for patients treated with immunotherapy versus chemotherapy *(63)*.

Pretreatment features associated with a shorter survival in the multivariate analysis were low Karnofsky performance status (<80%), high lactate dehydrogenase (>1.5 ↔ upper limit of normal), low hemoglobin (< lower limit of normal), high corrected serum calcium (>10 mg/dL), and absence of nephrectomy. These prognostic factors were used to categorize patients by risk into three different groups. The median time to death in the 25% of patients with zero-risk factors (favorable-risk) was 20 mo. Fifty-three percent of the patients had one or two of these prognostic features (intermediate risk), and the median survival in this group was 10 mo. Patients with three or more risk factors (poor risk), comprising 22% of the patients, had a median survival of 4 mo.

6. MONOTHERAPY VERSUS COMBINATION PROGRAMS

The combination of IFNα plus vinblastine showed a high-response proportion in several single-arm Phase II trials *(30)*. However, randomized trials failed to show improved survival, and the addition of vinblastine to interferon contributed gastrointestinal and hematologic toxicity *(17,52,61)*.

The combination of IFNα plus interleukin-2 (IL-2) was supported by preclinical studies showing synergistic actions. A large number of programs studied this combination, with wide variation in doses, schedules, and routes of administration. A review of 607 patients treated with IFNα plus IL-2 on 23 clinical trials showed a 19% response proportion, similar to that achieved with IL-2 alone *(64)*. The toxicities of these two agents in combination were additive, and a therapeutic benefit for the combination compared to IL-2 was not apparent *(64)*.

A randomized Phase II trial of high-dose IL-2 with IFNα versus high-dose IL-2 alone showed no difference in response proportion *(65)*. Moreover, in this randomized trial, increased toxicity was seen with the addition of IFNα to IL-2 compared to IL-2 alone *(65)*. A second randomized trial reported a higher response proportion for the combination of IL-2 plus IFNα compared to either agent given alone *(66)*. However, there was no benefit in survival associated with interferon plus IL-2 compared to interferon or IL-2 monotherapy, and the toxicity was more severe *(66)*.

Combination therapy with 5-fluorouracil, interferon, with/without IL-2 has been given in various schedules as inpatient and outpatient therapy. In several of these, high response proportions of between 30 and 40% were reported to interferon, IL-2, and 5-fluorouracil *(67–69)*. However, the combination studied by others has shown a response rate of less than 20%, characterized by a relatively short duration of response and formidable toxicity *(70–73)*.

The three-drug 5-fluorouracil-containing combination is currently being compared to interferon plus IL-2 in two randomized Phase III trials under way in Europe. Preliminary results from one showed no improvement in response to the combination of interferon, IL-2 plus 5-fluorouracil compared to interferon plus IL-2 *(74)*. In this trial, the response proportion to the three-drug regimen was 8% *(74)*. Inclusion of 5-flourouracil with IFNα and IL-2 contributes toxicity, and a conclusive statement on efficacy awaits further study in randomized trials.

Results of Phase II trials suggested that retinoids augmented the antitumor effect of IFNα against RCC *(75–78)*. However, a recently completed Phase III trial failed to show a benefit for the combination compared to IFNα alone *(79)*. To date, there has been no

Table 5
Phase III Trials of Interferon Monotherapy Against Interferon Combination Programs

Author	*Treatment*	*Patients*	*Survival Benefit for Combination*
Fossa *(61)*	IFNα versus IFNα plus vinblastine	178	No
Neidhart *(52)*	IFNα versus IFNα plus vinblastine	165	No
Negrier *(66)*	IFNα versus IL-2 versus IFN + IL-2	425	No
Motzer *(79)*	IFNα versus IFNα plus 13-cis- retinoic acid	283	None
Sagaster *(80)*	IFN versus IFNα plus coumarin and cimetidine	148	None
DeMulder *(81)*	IFNα versus IFNα plus IFNγ	102	None
Creagan *(83)*	IFNα versus IFNα plus aspirin	176	None

sufficiently powered randomized Phase III trial showing a survival benefit for combination therapy compared to single-agent interferon (Table 5) *(52,61,66,79–83)*. Each program showed promise in Phase II trials, and reaffirms the necessity to conduct Phase III trials to prove efficacy of novel treatment programs.

7. SECOND-LINE THERAPY

A recent trial addressed the potential role for treatment with IFNα in patients with advanced RCC following progression to treatment with IL-2 *(84)*. Patients were selected from the CRECY trial, a randomized comparison of IFNα, IL-2, or combination therapy *(66)*. Forty-eight patients were treated with IFNα as salvage therapy following treatment with IL-2 *(84)*. One patient achieved a partial response (2%) of 18 mo duration, with similar toxicity of that observed in first-line treatment *(84)*. The authors concluded that crossover treatment after failure of IL-2 is not effective therapy in patients with advanced RCC.

8. ADJUVANT THERAPY

Twenty to 30% of patients with completely resected RCC relapse after a radical nephrectomy *(85–87)*. Predictors of relapse include renal vein involvement and nodal metastasis(es) *(85–87)*. IFNα given as adjuvant therapy following complete resection of RCC with renal vein or nodal involvement has been compared to observation in three randomized trials *(88–90)*. These studies are discussed in Chapter 13. Results of IL-2 given as adjuvant therapy in a Phase III trial have not been reported. Therefore, standard care remains observation following nephrectomy, because no recognized systemic therapy reduces the likelihood of relapse.

9. DIRECTIONS

Metastatic RCC remains a disease highly resistant to systemic therapy. Small numbers of patients exhibit complete or partial responses to interferon and/or IL-2, but most patients do not respond and there are few long-term survivors. Therefore, the identification of new agents with better antitumor activity against metastases remains the highest priority of clinical investigation in this refractory tumor. Efforts of continued investigation for interferon focus on studies of combination therapy. Randomized trials are under way to assess the relative efficacy of IFNα in combination with IL-2 and 5-fluorouracil. Combination studies with angiogenesis inhibitors and a monoclonal antibody to epidermal growth factor *(91)* are planned.

Second generation IFNα compounds are currently in clinical trials. Two being studied are pegylated, comprised of polyethylene glycol conjugated to IFNα. The prolonged half-life of elimination associated with pegylated IFNα-2a compound allows for weekly administration, which may represent an advantage over the more frequent administration required for currently available preparations. The relative efficacy and toxicity compared to standard recombinant preparations is being investigated.

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21 Combination Therapy for Treatment of Advanced Renal Cell Carcinoma

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Contents

1. INTRODUCTION

Metastatic renal cell carcinoma (RCC) is a poor prognosis disease, with a 5-yr survival below 5%; as yet, no standard therapy regimen has been established *(1,2)*. In advanced disease, the administration of chemotherapy alone is of minor benefit most likely because of high p-glycoprotein-expression (MDR-1) *(3–5)*. Modulation of MDR-1 with dexverapamil, cyclosporin, quinidine, or acrivastin to increase the therapeutic effect of vinblastine rendered no significant benefits *(6–9)*. Phase II studies with newer substances, such as gemcitabine, mebaron, liposomal doxorubicin, topotecan, taxotere, or echinomycin yielded no significant therapeutic effects as well *(10–15)*. Although the effectiveness of vinblastine and 5-fluorouracil (if applied as single agents) is approximately 10%, these agents are more often used in combined chemoimmunotherapy protocols.

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Based on experimental tumors in Syrian golden hamster models *(16)*, hormonal manipulations were felt to be justified in advanced renal cancer patients. Patients were treated with a variety of hormone agonists and antagonists, such as corticosteroids, progesterones, and antioestrogens. These agents showed no significant therapeutic efficacy in RCC *(17)*.

Tamoxifen is a nonsteroidal antioestrogen successfully administered in breast cancer patients *(18)*. The clinical efficacy of tamoxifen in advanced RCC was found to be non-significant *(16,19,20)*.

2. CELLULAR AND TARGETED IMMUNOTHERAPIES: LYMPHOKINE-ACTIVATED KILLER CELLS, TUMOR-INFILTRATING LYMPHOCYTES, AUTOLOGOUS TUMOR CELLS, GENE THERAPY, AND MONOCLONAL ANTIBODIES

In the treatment of patients with RCC, cellular immunotherapy with tumor-infiltrating lymphocytes (TIL) or lymphokine-activated killer cells (LAK) has been used in combination with interleukin 2 (IL-2). However, there has been no significant improvement compared to the administration of IL-2 alone (*21–24*; Table 1). At present, genetically engineered TILs serve primarily scientific, i.e., investigational purpose with gene-marking of reinfused autologous lymphocytes *(25)*.

Active specific immunotherapy (ASI) uses preparations of autologous or allogeneic tumor cells to induce a specific antitumor directed T-cell response *(26)*. Its role in the treatment of metastatic RCC has not been established, but may be important in the adjuvant treatment of locally advanced renal cancer *(27)*. A first prospective-randomized clinical trial with a follow-up of 5 yr using autologous tumor cells and BCG showed no significant difference in progression free- and overall survival compared to an untreated control group, however *(28)*.

In the treatment of tumor patients, the genetically modified tumor cell preparations increase the antitumor activity whereby a specific immunogenic response to patient tumor cells is induced by gene transfer of IL-2, costimulating molecules like B-7.1 and HLA-molecules, respectively *(29–33)*. Immunotherapy with specific immunogenic peptides has become possible with the cloning of defined tumor-antigens *(34,35)*. A tumor, i.e., patient-specific HLA-A-2 molecule, altered by point-mutation *(36)*, and the HLA-B7-restricted RAGE-1 antigen, expressed in different tumors, were discovered *(37)*. Monoclonal antibodies continue to be the subject of clinical studies. At present, they play no role in the treatment of patients with RCC *(38,39)*.

3. COMBINATION IMMUNOTHERAPY WITH CYTOKINES AND OTHER GROWTH FACTORS

Patients with RCC have been treated with a variety of different cytokines. Among these, the interferons, especially interferon-α2 (IFNα2), and IL-2 are of major importance *(20,40)*. For confirmation of the clinical efficacy of immunomodulating therapy, further prospective-randomized trials will have to be conducted. Other cytokines such as IL-4 *(41)*, IL-6 *(42)*, the hematopoetic growth factor GM-CSF alone or in combination with IL-2 *(43,44)*, as well as GM-CSF in ex vivo stimulation of TILs, together with IL-2 *(45)* continue to be under investigation. Immunotargeting represents an experimental approach

Table 1
Results of Different Studies with IL-2 as Single Agent in Metastatic RCC

IL-2-application *Author*	*Totals*	*Complete Remission (%)*	*Partial Remission (%)*
IVB, high-dose:			
Bukowski *(71)*	41	1 (2)	4 (10)
Yang et al. *(55)*	65	2 (3)	11 (17)
Rosenberg et al. *(72)*	149	10 (7)	20 (13)
Fyfe et al. *(49)*	255	12 (5)	24 (9)
All	510	25 (5)	59 (12)
CIV, moderate-dose:			
Sosman et al. *(73)*	17	0 (0)	3 (18)
Negrier et al. *(74)*	32	2 (6)	4 (13)
West et al. *(75)*	8	0 (0)	1 (13)
Perez et al. *(76)*	12	0 (0)	2 (17)
Masse et al. *(77)*	51	2 (4)	6 (12)
Geersten et al. *(78)*	30	2 (7)	4 (13)
Lopez et al. *(79)*	29	0 (0)	4 (14)
Whitehead et al. *(80)*	43	0 (0)	4 (9)
Law et al. *(21)*	34	1 (3)	2 (6)
All	256	7 (3)	30 (12)
SC, low-dose:			
Marumo et al. *(81)*	13	2 (15)	1 (8)
Lissoni et al. *(82)*	13	0 (0)	4 (31)
Guida et al. *(83)*	9	0 (0)	1 (11)
Buter et al. *(84)*	46	2 (4)	7 (15)
All	81	2 (3)	13 (16)
IL-2 + LAK:			
Rosenberg et al., ivb *(85)*	36	4 (11)	8 (22)
Fisher et al., ivb *(86)*	32	2 (6)	3 (9)
Mittelman et al., ivb *(87)*	12	0 (0)	1 (8)
Negrier et al., civ *(74)*	51	5 (10)	9 (18)
Clark et al., ivb + civ *(88)*	13	0 (0)	2 (15)
Parkinson et al., ivb + civ *(89)*	47	2 (4)	2 (4)
Weiss et al., ivb *(90)*	46	3 (7)	6 (13)
Thompson et al., civ *(91)*	42	4 (10)	10 (24)
Foon et al., civ *(92)*	23	2 (9)	4 (17)
Rosenberg et al., ivb *(93)*	46	7 (15)	8 (17)
Law et al., civ *(21)*	32	0 (0)	1 (3)
Gold et al., civ *(23)*	123	9 (17)	14 (11)
All	503	38 (7)	68 (13,5)

LAK = Lymphokine-activated killer cells; IVB = intravenous bolus; CIV = continuous intravenous infusion; SC = subcutaneous.

utilizing conjugated cytokines that ultimately bind to relevant receptors expressed on RCC tumor cells. This method can be used with toxic agents, such as the pseudomonas-exotoxin A and a cytokine *(46)*. These therapeutic strategies are still within the experimental phase.

Table 2
Results of Different Studies with sc IL-2/IFNα in Metastatic RCC

Author	Totals	Complete Remission (%)	Partial Remission (%)
Atzpodien et al. *(58)*	34	4 (12)	6 (17)
Sznol et al. *(94)*	23	0	5 (22)
Ratain et al. *(95)*	16	0	4 (25)
Vogelzang et al. *(96)*	42	1 (2)	4 (10)
Negrier et al. *(97)*	34	1 (3)	6 (18)
Ravaud et al. *(98)*	38	1 (3)	6 (16)
Atzpodien et al. *(40)*	152	9 (6)	29 (19)
Facendola et al. *(99)*	50	6 (12)	3 (6)
Piga et al. *(100)*	20	1 (5)	2 (10)
Buizo et al. *(101)*	20	1 (5)	3 (15)
Bukowski *(69)*	36	3 (8)	3 (8)
Karp et al. *(102)*	14	1 (7)	0 (0)
All	479	28 (6)	71 (15)

4. IL-2 AND ACTIVATED LYMPHOCYTES

IL-2 is a potent immunomodulating cytokine inducing secondary mediators and cellular reactions responsible for antitumor activity *(47,48)*. The proven effectiveness of the high-dose intravenous (iv) IL-2 therapy in metastatic RCC caused the Federal Drug Administration (FDA) to introduce this therapeutic regimen for clinical use. The decision was based on a study of 255 patients with objective response rates of 14% (12 CRs and 24 PRs) and a median response duration of 30.6 mo (range, 3–95 mo) *(49,50)*. Different methods of applying IL-2 have been described: (1) a high-dose iv bolus application developed by Rosenberg *(51)*; (2) the moderate-dose continuous iv infusion according to West *(52)*; and (3) the low-dose subcutaneous (sc) injection *(53)* used predominantly in Europe. IL-2 can also be used as an inhalative topical agent in pulmonary metastasis of RCC *(54)*, as well as for ex vivo stimulation of LAK or for the cultivation of TILs. These modes of systemic application have variable side effects and overall tolerability. So far, the study results of the different forms of administering IL-2 demonstrate no significant difference in therapeutic effectiveness. However, a direct comparison between the low-dose sc application and the high dose iv bolus application of IL-2 has yet to be performed *(47,48,55)*. Table 1 shows the results of the different studies of the high-dose bolus application, the moderate continuous iv and the low-dose sc IL-2 therapy in patients with metastatic RCC. A combination of cellular therapy using LAK and IL-2 shows no improvement of treatment results *(21)* over cytokines alone.

5. IFNα AND IL-2

The therapeutic efficacy of IFN-α2 alone shows objective remissions in up to 15%. Before establishing recombinant human interferons in the treatment of RCC, the highly purified leukocyte interferon of Cantell *(56)* and the partially purified lymphoblastoid interferon originating from cell lines of human Burkitt-lymphoma have been used. The joint administration of IL-2, together with IFNα2 shows a clear improvement in treat-

Table 3
Results of Combination Therapies with 13-*cis*-Retinoic Acid

Author	*Therapy*	*Totals*	*Complete Remission (%)*	*Partial Remission (%)*
Atzpodien et al. *(20)*	sc IL-2/sc IFNα/iv 5-FU, iv VBL/ po 13-C-RA	24	4 (17)	6 (25)
Motzer et al. *(64)*	sc IFNα/po 13-C-RA	44	3 (7)	10 (23)
Jacobs et al. *(103)*	sc IFNα/po 13-C-RA	17	1 (6)	3 (18)
Stadler et al. *(61)*	sc IL-2/sc IFNα/po 13-C-RA	47	1 (2)	7 (15)
All		132	9 (7)	26 (20)

5-FU = 5-fluorouracil, VBL = Vinblastine, 13-C-RA = 13-*cis*-retinoid acid, sc = subcutaneous, po = peroral.

ment outcome (Table 2). Both agents have been simultaneously employed in an out-patient treatment regimen with an objective response rate of 36% *(53)*. The safety and clinical efficacy of sc immunotherapy using cytokines (IL-2, INFα2) has been established *(57,58)*. The sc use of recombinant IL-2 and recombinant IFNα2 has yielded promising results and has been established in the treatment of patients with metastatic RCC. Currently, IFNα2 and IL-2 have been confirmed as standard systemic therapy of advanced RCC.

6. CYTOKINES AND 13-*CIS*-RETINOIC ACID

The anticancer effects of retinoids have been evaluated as primary therapy for advanced diseases (e.g., AML, MDS, lung, skin, and bladder cancer), as primary or secondary cancer chemopreventive drugs, and in combination with other agents *(59)*. Promising clinical results were reported when p.o. 13-*cis*-retinoic acid (13-C-RA) was combined with immunotherapy and chemoimmunotherapy in RCC patients *(20,60–64)*. In contrast, retinoids seem to be ineffective as single agents in the treatment of metastatic RCC *(65)*. Combined therapy regimens including INFα2 and 13-C-RA are the subject of recent studies (Table 3). Therapy was given in the out-patient setting; 13-C-RA produced no significant side effects and/or toxic deaths *(20)*.

7. COMBINED CHEMOIMMUNOTHERAPY

Through the combination of the different concepts, such as chemotherapeutic and immunotherapeutic strategies, an additive or synergistic effect can be gained employing the various mechanisms of action without significantly enhancing the adverse side effects. Combining IFNα2 with chemotherapy, such as vinblastine or 5-fluorouracil, shows no significant improvement of the treatment results when compared with IFNα2 given alone *(66,67)*.

Improved results can be obtained using combination therapies with IL-2, IFNα2, and 5-fluorouracil, with or without additional vinblastine *(20,68)*. Table 4 presents an overview. 13-C-RA also seems to have a favorable effect in the treatment of advanced RCC, when added to combined chemoimmunotherapy regimens (Tables 3 and 4; *60,61*).

8. CLINICAL PREDICTORS FOR COMBINATION THERAPIES

The comparison of clinical studies must be viewed critically because the therapeutic response rates and the overall prognosis varies individually from patient to patient and

Table 4
Results of Biochemotherapy in Metastatic RCC

Author	*Therapy*	*Totals*	*Complete Remission (%)*	*Partial Remission (%)*
Sella et al. *(104)*	civ IL-2/sc IFNα/iv 5-FU	19	3 (16)	6 (31)
Atzpodien et al. *(20)*	sc IL-2/sc IFNα/iv 5-FU, iv VBL/ po 13-C-RA	24	4 (17)	6 (25)
Lopez Hänninen et al. *(57)*	sc IL-2/sc IFNα/iv 5-FU	120	13 (11)	34 (28)
Joffe et al. *(105)*	sc IL-2/sc IFNα/iv 5-FU	54	0 (0)	9 (17)
Hofmockel et al. *(106)*	sc IL-2/sc IFNα/iv 5-FU	34	3 (9)	10 (29)
Atzpodien et al. *(68)*	sc IL-2/sc IFNα/iv 5-FU[a]	41	7 (17)	9 (21)
Ellerhorst et al. *(70)*	sc IL-2/sc IFNα/iv 5-FU	52	4 (8)	12 (23)
Pectasidos et al. *(107)*	sc IL-2/sc IFNα/iv VBL	31	4 (13)	8 (26)
Tourani et al. *(108)*	sc IL-2/sc IFNα/iv 5-FU	62	1 (2)	11 (17)
All		437	39 (9)	105 (24)

5-FU = 5-fluorouracil, VBL = vinblastine, 13-C-RA = 13-*cis*-retinoid acid, sc = subcutaneous, po = peroral, civ = continuous intravenous infusion

[a] Controlled, prospective-randomized analysis compared to tamoxifen

Table 5
Independent Prognostic Variables in Patients with Metastatic RCC
(Lopez Hänninen, et al., 1996)

Prognostic Variable[a]	*p Value*	*Risk Score/Rating*[b]
Erythrocyte sedimentation rate (greater than 70 mm/after 1 h)	0.0001	2
Lactic dehydrogenase (greater than 280 U/L)	0.0001	2
Neutrophilic granulocytes (greater than 6000/μL)	0.006	1
Hemoglobin (less than 100 gm/L)	0.005	1
Extrapulmonary metastases only	0.005	1
Bone metastases	0.004	1

[a]Assessed by multivariate analysis. Factors also evaluated and rendered not independent by multivariate analysis included performance status, weight loss, time from initial diagnosis, prior nephrectomy, prior chemotherapy or immunotherapy, number of tumor sites, lung metastases, liver metastases, local relapse, lymphatic metastases, tumor size, dose intensiy, leukocytes, gamma GT, alkaline phosphatase, and C-reactive protein.

[b]Individual risk was defined as cumulative risk score, that is function of the sum of six independent variables. Patients were assigned to low risk (score 0), intermediate risk (score 1 to 3), or high risk (score 4 or greater).

seems to be influenced by different clinical predictors *(69)*. We established a risk-stratification employing retrospective analysis of 215 consecutive patients treated with IL-2-based immunotherapy between 1988 and 1993 *(57)*. This multivariate analysis, demonstrated elevated erythrocyte sedimentation rate above 70 mm after 1 h, as well as increased serum lactic-dehydrogenase above 280 U/L present independent and major predictors of survival ($p = 0.0001$). Neutrophilic granulocytes of more than 6000/μL, a hemoglobin below 100 gm/L, and extrapulmonary metastases, or the presence of bone metastases were additional statistically independent ($p = 0.006$) predictors (Table 5). Using a cumulative risk score with the aforementioned independent variables, three risk groups were identified whereby patients with a low, intermediate, and high risk had median survivals of 39.4 mo, 15 mo, and 6.2 mo, respectively.

Even though the prognosis of patients with metastatic RCC is poor, objective and durable remissions occur in approximately one-third of patients using systemic cytocine-based combination therapies *(70)*. As of today, it appears that combination chemoimmunotherapy is far more effective than single-agent cytokine treatment and chemotherapy, respectively. Further studies will have to be designed to improve the therapeutic index and cost effectiveness of systemic combination therapy in metastatic RCC.

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22 Monoclonal Antibodies in Advanced Renal Cell Carcinoma

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Contents

1. ROLE OF THE IMMUNE SYSTEM IN RENAL CANCER

The modern era of cancer immunology began in 1943 with Ludwig Gross' *(1)* observation that inbred mice could be immunized against tumor transplants from syngeneic animals. Subsequently, Prehn and Main *(2)* demonstrated that immunization with normal tissue did not afford protection implying that cancers possessed tumor-related or tumor-specific antigens that were responsible for inducing the protective effect. Integrating the evolving knowledge, Burnet coined the term "immune surveillance" *(3)*. He proposed that cancers expressed aberrant antigens that would allow their detection and elimination by the host's immune system. Attempts followed to define the recognized tumor antigens and to discern the reasons why immune surveillance sometimes fails and allows the development of a cancer.

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While cancer immunologists continue to wrestle with these questions, there is in renal cancer, at least indirect evidence implying a role of the immune system in the natural history of this disease. First, spontaneous regression occurs more often in renal cancer than in any other solid tumor *(4–7)*, albeit still quite rarely. Along similar lines, Oliver *(8)* reported a series of 73 patients with metastatic renal cancer whom he observed without therapy until their disease progressed. This study provided an excellent view of the natural history of metastatic renal cancer as well as a baseline for comparison to therapy trials. In this study, patient's disease was scored by standard oncologic response criteria: complete or partial response, stable, or progressing disease. Oliver found that without treatment, 4% of patients had a complete response (CR), 3% had a partial response (PR), and 5% had a prolonged period (>12 months) of stable disease. Again, perhaps some of the "responses" in the absence of treatment are a manifestation of the immune system at work.

Yet another piece of indirect evidence are the many cases where metastases develop 10, 20, or even more years after resection of an apparently localized cancer. In these cases, one must presume that the cancer had metastasized prior to the resection, but that the metastatic foci had remained in check by the immune system.

Finally, there are the dramatic cases of regressions occasionally seen when patients are treated with immunotherapy such as interleukin-2 (IL-2) *(9)*. The ability of an agent, which physiologically activates lymphocytes to yield significant antitumor effects, is certainly consistent with a role of the immune system in the natural history of renal cancer.

2. NORMAL AND NEOPLASTIC KIDNEY ANTIGENS DEFINED BY MONOCLONAL ANTIBODIES

The search for tumor-related antigens was given an enormous boost by the development of hybridoma technology *(10)* in 1975. The profound impact of this technology on all aspects of immunological investigation and on the development of diagnostic immunoassays (of which Prostate Specific Antigen is but one of many examples) is reflected in the awarding of the Nobel Prize to the discoverers of this technology. Application of this technology to the study of cancer, while not resulting in definition of true tumor-specific antigens in the absolute sense, has provided many useful antibody probes, new insight into cancer biology and the promise of developing diagnostic and therapeutic advances. As of this writing, there are 8 mAbs approved by the U.S. F.D.A. for treatment of a variety of diseases. MAbs comprise 20% of all agents currently in phase III testing. In oncology, mAbs are approved for imaging (including prostate cancer) and for treatment of non-Hodgkin's lymphoma (NHL) and breast cancer. Additional antibodies are anticipated to be approved later in 1999 for NHL and acute myelogenous leukemia.

In the study of renal cancer, investigation by various laboratories has provided numerous mAb probes with a high degree of specificity for kidney-related antigens. These probes can be readily used to define the presence or absence of their respective antigens either in fresh tissue specimens or in vitro. In many cases, the mAb has allowed cloning, sequencing and identification of the detected antigen's gene.

Some of the important mAbs and the antigens they helped define in the study of renal cancer are listed in Table 1. Expression of these antigens is, in most cases, not only quite specific for the kidney, but also for particular segments of the nephron. This allows development of a so-called "antigenic map" of the kidney shown in Fig. 1. This informa-

Table 1
Monoclonal Antibodies and Defined Antigens in Normal Kidney and Renal Cancer

Monoclonal Antibody	*Defined Antigen*	*Site of Antigen Expression*
T138	gp25	Vascular endothelium
J143 (URO-1)	gp140, 120, 30	Glomerular epithelium
C5H	p115	Glomerulus
S22	gp115	Bowman's capsule
D5D	not defined	Bowman's capsule
AJ8 or J5	neutral endopeptidase (NEP)	Glomerulus, proximal tubule
S4 (URO-2)	aminopeptidase A (APA)	Glomerulus, proximal tubule
F23 (URO-3)	aminopeptidase N (APN)	Proximal tubule
T43 (URO-10)	gp85	Proximal tubule-convoluted segment
F31 (URO-8)	acidic lipid	Proximal tubule-straight segment
S27 (URO-4)	dipeptidyl peptidase (DPP) IV	Proximal tubule, loop of henle
A6H	not defined	Proximal tubule
10.32	gp90, Tamm-Horsfall protein	Loop of Henle, distal tubule
C26	gp40	Distal tubule, collecting duct
T16 (URO-5)	gp48, 42	Distal tubule, collecting duct
anti-A, B, O(H)	blood group antigens	Collecting duct
G250	Carbonic anhydrase (CA) IX	Clear cell RCC only

tion provides the antigenic or molecular phenotype of the various cells that comprise the normal kidney. This ability is analogous to using antisera to "type" red blood cells [e.g., A, B, O(H), Rh, and so on] based on antigenic expression. Similarly, mAbs are used to subclassify histologically indistinguishable lymphocytes into T (CD3 antigen-positive) or B cells ($CD3^-$) and further subclassify T cells into the functional subcategories of helper ($CD3^+/CD4^+/CD8^-$) or cytotoxic ($CD3^+/CD4^-/CD8^+$) T cells. In the kidney, proximal convoluted tubular cells, for example, have the molecular phenotype URO-10^+/ aminopeptidase A^+/aminopeptidase N^+/neutral endopeptidase$^+$/dipeptidyl peptidase IV^+/ Lewis x^+/URO-8^-/ABH^-, while cells of the collecting duct express virtually the reciprocal pattern of expression, URO-10^-/aminopeptidase A^-/aminopeptidase N^-/neutral endopeptidase $^-$/dipeptidyl peptidase IV^-/Lewis x^-/URO-8^-/ABH^+ *(11–14)*.

Definition of the antigenic or molecular phenotype of the various cell types, which comprise the normal adult nephron, provides a reference point for further studies and allows determination of cell type outside of the normal anatomical architecture of the nephron—for instance, in tissue culture or in a kidney cancer. For example, one can define the phenotype of fetal kidney cells to study renal development. Fetal proximal tubule cells, unlike the adult, express both the URO-8 and URO-10 antigens, whereas in the mature proximal tubule, these antigens are reciprocally expressed *(11)*. Cells of the convoluted proximal tubule are URO-10^+/URO-8^-, whereas cells of the straight segment of the proximal tubule are URO-10^-/URO-8^+. These molecular differences, therefore, allow one to distinguish cells from the fetus or adult or from the convoluted or straight proximal tubule.

Similarly, one can use these probes to study the histogenesis of renal cancer. Study of the molecular phenotype of renal cancers confirms earlier ultrastructural and conventional immunological data that the vast majority of renal cancers derive from the proximal tubule *(11)*. With mAb probes, this classification can be taken one step further

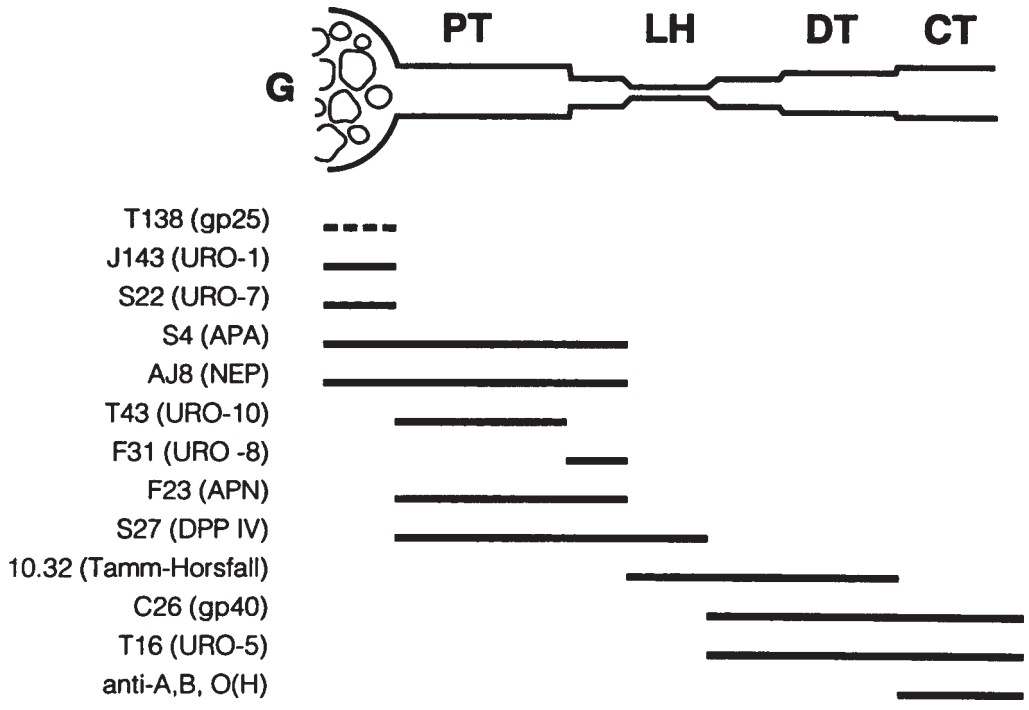

Fig. 1. Antigenic map of the human nephron. The nephron is schematically represented below which the sites of antigen expression are indicated by the solid bars. G, glomerulus; PTc, convoluted proximal tubule; PTs, straight proximal tubule; LH, Loop of Henle; DT, distal tubule; CT, collecting duct. The definitions of the antigens are found in Table 1.

because of the ability of mAbs to distinguish cells from the convoluted or the straight portion of the proximal tubule. In a study of 200 renal cancers, approximately 30% have a molecular phenotype consistent with derivation from the convoluted PT; 20% appear derived from the straight segment and 50% have the phenotype of the less well-differentiated, fetal proximal tubular cells *(11)*. It remains to be determined whether these different subsets have clinical significance.

One can also apply the defined antigenic phenotypes of normal kidney cells to the study of normal and neoplastic renal epithelium growing in tissue culture. The ability to readily grow short-term cultures of normal kidney cells and establish immortal lines of renal cancers is an experimental advantage in renal cancer unique in the study of human cancers *(15)*. This experimental advantage is compounded by the fact that the molecular phenotyping of normal renal epithelial cultures reveals that these cells are derived from the proximal tubule—the same cell type that transforms to renal cancer. The ability to grow a human cancer cell in tissue culture alongside its normal counterpart—both from the same patient—is unique to renal cancer and provides an unparalleled opportunity for the study of neoplastic transformation at a molecular level.

Because virtually every clear cell renal cancer we have studied with these mAb probes demonstrates a pattern consistent with proximal tubular derivation, it is not uncommon for one or more of the group of proximal tubular antigens to be deleted. Therefore, this creates a series of molecular subtypes of renal cancer. Additionally, there exists a direct correlation between the number of antigenic markers expressed and the degree of differ-

entiation of the cancer. Perhaps scoring the antigenic expression of a patient's cancer could provide an objective means of grading renal cancers. Furthermore, we have seen that the greater the number of antigens deleted, the more rapid the progression and the more grave the prognosis. However, it remains to be determined whether antigen loss could function as an independent prognostic variable or whether our preliminary observations are simply a reflection of tumor grade. Further follow-up studies of a larger number of patients will be necessary to clarify this point.

It is becoming increasingly clear that advances in molecular biology are moving the surgical pathologist beyond his or her microscope toward an era of molecular pathology. Clearly, molecular probes such as mAbs or oligonucleotides can discriminate that which is not apparent at the level of the microscope. Increasingly, it is being recognized that there are multiple subtypes of renal cancer that can be subclassified at the molecular level. As these subtypes are being elucidated, it is apparent that these distinctions have clinical relevance. One example where molecular differences are emerging to resolve different clinical findings is demonstrated by the previously recognized morphological entities of papillary (chromophilic) and nonpapillary clear cell renal cancers. The differences go beyond their morphology. It has long been recognized that the substantial majority (80%) of renal cancers have a clear or granular cell histology, whereas a minority of renal cancers (10–15%) have a papillary growth pattern. It has been similarly appreciated that the clear or granular cell type was typically hypervascular, whereas papillary renal cell carcinoma (RCC) was typically hypovascular. It is only now becoming apparent, with the elucidation of the molecular genetics of renal cancer, that different genetic defects underlie these two renal cancer subtypes. Nonpapillary clear cell renal cancers have a defective von Hippel-Lindau disease (VHL) gene *(16)*. Papillary (chromophilic) renal cancers have normal VHL genes but overexpress the *c*-MET oncogene *(17)*. At least one mAb, G250, can distinguish the cell types as well: the "G250" antigen (now known to be carbonic anhydrase IX; *see* Subheading 3.) is expressed by the clear/granular cell type but neither by normal kidney epithelia nor by papillary RCC *(18)*. Recently, the explanation for this finding has become apparent. Ivanov et al. *(19)* found that the normal VHL gene product (*p*VHL) suppresses synthesis of CA IX. Therefore, in both normal proximal tubule cells and in papillary RCC with normal *p*VHL, no CA IX is detectable. In clear cell RCC, however, the mutated VHL gene product no longer suppresses CA IX, resulting in its expression. Because the details of specific VHL mutations and their relationship to CA IX expression remain to be defined, CA IX expression by RCC cells, detectable with the G250 mAb, may serve as a surrogate marker of VHL mutation. A similar relationship also explains the differential vascularity of clear cell and papillary RCC in that loss of *p*VHL function (in clear cell RCC) leads to VEGF expression and resulting hypervascularity. With normal *p*VHL in papillary RCC, hypervascularity is not seen.

3. CLONING THE G250 ANTIGEN

In view of the induction/ upregulation of G250 antigen in clear cell RCC, its restricted tissue expression and, therefore, its potential as therapeutic target, we set out to molecularly identify the cDNA encoding the G250 antigen. Screening of a cDNA expression library by immunohistochemical means *(20)*, resulted in the isolation of a cDNA clone of 1534 bp, designated pMW1. This cDNA was used as a probe for Northern blot analysis of mRNA isolated from RCC cell lines (mAbG250$^+$ and mAbG250$^-$), surgical specimens (RCC and normal kidney obtained from the same patients), and normal human organs

(fetal kidney, adult kidney cortex, and kidney medulla, liver, colon, placenta, muscle, prostate, spleen). After hybridization under stringent conditions a single 1.5-kb transcript was detected in mAbG250-positive cell lines and RCC specimens. Complete correlation with respect to G250-mRNA expression and protein expression, as detected by mAbG250 immunohistochemistry, was observed. A solitary exception was the SK-RC-16 cell line that shows very low G250 mRNA levels, with undetectable antigen expression was by mAbG250 immunohistochemistry. G250 mRNA expression levels in fresh RCC was remarkably similar in all cases examined and comparable to the highest mRNA levels observed in RCC cell lines. No transcript was detected in mAbG250-negative cell lines, normal kidney specimens, or any normal human organ investigated. Transfection of mAbG250-negative cell lines resulted in a conversion to the G250-positive phenotype, confirming that the cDNA encoded for G250 protein.

Southern blot analysis of human chromosomal DNA showed that the gene encoding for G250 is present in the human genome as a single copy gene of approximately 7.2 kb. Fluorescent *in situ* hybridization (FISH) was performed to assign the G250 gene to chromosome 9*p*12–13. Amplification of G250-encoding DNA was not observed in any of the surgically obtained RCC specimens or RCC cell lines.

The proposed open reading frame encodes for a protein of 459 amino acids, with a predicted molecular weight of approximately 49.7 kD. Homology analysis showed that the G250 protein can be divided into regions containing a signal peptide (aa 1–37), a carbonic anhydrase domain (aa 134–391) and a hydrophobic transmembrane region of 20 aa and a C terminus of 25 aa. Computer-assisted comparison with the EMBL database revealed partial homology with MN, a recently cloned human tumor-associated protein, originally identified in HeLa cells using mAb M75 *(21)*, and complete homology with a revised MN version *(22)*. The G250 sequence has been deposited under accession number DS 35472.

Western blots probed with M75 mAb after mAbG250 affinity purification revealed reactivity with a protein of 63-kD molecular weight, compared to the predicted molecular weight of 49 kD. After correction for the introduced modifications (*his-myc* tag), this points to posttranslational modification of 10 kD. Indeed, in RCC three proteins of 49, 52, and 59 kD, most likely representing the unmodified, intermediate modified, and final product could be detected by M75.

Characterization of the genomic organization of the G250 gene revealed that the gene consists of 11 exons and 10 exons, identical to the MN gene structure *(22)*. With the exception of the first exon, all exons are small. Splice donor and acceptor sequences conformed to consensus splice sequences. No differences were observed between the cDNA and genomic sequence, with the exception of an A-G transition in codon 33 in exon I (nucleotide 106), leading to the change of methionine to valine. The sequence surrounding this ATG does not conform to a Kozak consensus sequence and it is unlikely that this methionine functions as an alternative start site. SSCP analysis of exon I showed no differences between RCC and corresponding normal kidney tissue in 10 cases examined. Thus, this transition seems to represent a naturally occurring polymorphism. Both alleles are functional since homozygous cell lines showed G250 expression, irrespective of codon 33 use.

G250 transcripts were undetectable in normal kidney specimens (adult and fetal) by Northern analysis or RT-PCR. Additionally, hybridization of pMW2 with a cDNA library constructed from normal kidney failed to identify any clone with sequence homology to G250. These observations indicate that G250 transcription is induced or dramat-

ically upregulated upon malignant transformation of proximal tubular cells, and not involved in renal organogenesis. It also indicates that mAbG250 does not recognize a unique epitope on a (kidney) differentiation-antigen, but reacts with an aberrant expressed protein unique for RCC.

4. POTENTIAL USE OF MONOCLONAL ANTIBODIES IN VIVO FOR DIAGNOSIS AND THERAPY

mAbs are undergoing clinical investigation in many tumor types because of their now proven ability to specifically target cytotoxicity to tumor sites while sparing normal tissues. mAbs are now FDA-approved for treatment of NHL and breast cancer. A radiolabeled mAb and a cytotoxin-conjugated mAb for treatment of NHL and acute myelogenous leukemia, respectively, are expected to be approved in the near future.

In RCC, clinical experience with the use of mAbs is most extensive with mAb G250 initially developed by Oosterwijk et al. *(23)*. As indicated above, mAb G250 recognizes carbonic anhydrase IX, whose expression is suppressed in normal renal epithelium by *p*VHL. In clear cell RCC, loss of *p*VHL function leads to expression of CA IX and, therefore, reactivity with mAb G250. mAb G250 is not tumor-specific in the absolute sense as the mAb demonstrates some reactivity with normal gastric mucosal cells and with biliary ductules.

Clinical studies with radiolabeled mAb G250 in renal cancer patients *(18,24–27)*, has demonstrated the ability to selectively and specifically deliver mAb to renal cancer sites. G250 can successfully target and image both primary and metastatic RCC including both bone and soft tissue metastasis (Fig. 2). Tissue biopsies of approximately 30 imaged lesions were taken from 23 patients. All of these imaged lesions were pathologically documented to represent sites of renal cancer. That is, there were no false positive scans. Quantitative analysis of tissue samples demonstrated very specific mAb localization to tumor compared to normal, with peak ratios of tumor:serum of 178:1, tumor:normal kidney of 285:1 and tumor:liver of 92:1 at one week after mAb administration *(18)*. In addition, 10 of 48 (21%) patients studied *(18,27)* could be shown to have imaged sites not suspected on the basis of conventional imaging techniques (e.g., CT, MRI, or bone scan). In many of these cases, these sites were pathologically confirmed, and the finding of unsuspected sites often had significant impact on treatment decisions. For example, two patients with presumed solitary bone metastasis who were referred for surgery, were found to have additional lesions, thereby sparing the patients a needless operation. In at least two other cases, unsuspected sites imaged by the mAb were resected and proven to be metastatic RCC. Had they not been imaged by the mAb, they would have been left *in situ*.

5. PHASE I/II RADIOIMMUNOTHERAPY WITH MURINE ^{131}I-LABELED G250

We carried out a Phase I/II radioimmunotherapy at Memorial Sloan-Kettering Cancer Center to determine the maximum tolerated dose (MTD_A) and therapeutic potential of ^{131}I-G250 *(26)*. Thirty-three patients with measurable metastatic RCC were treated. Groups of at least three patients received escalating amounts of ^{131}I (30, 45, 60, 75, 90 mCi/m^2 ^{131}I) labeled to 10 mg mG250, administered as a single intravenous infusion. Fifteen patients were studied at the maximum tolerated dose of activity (MTD_A). MTD_A

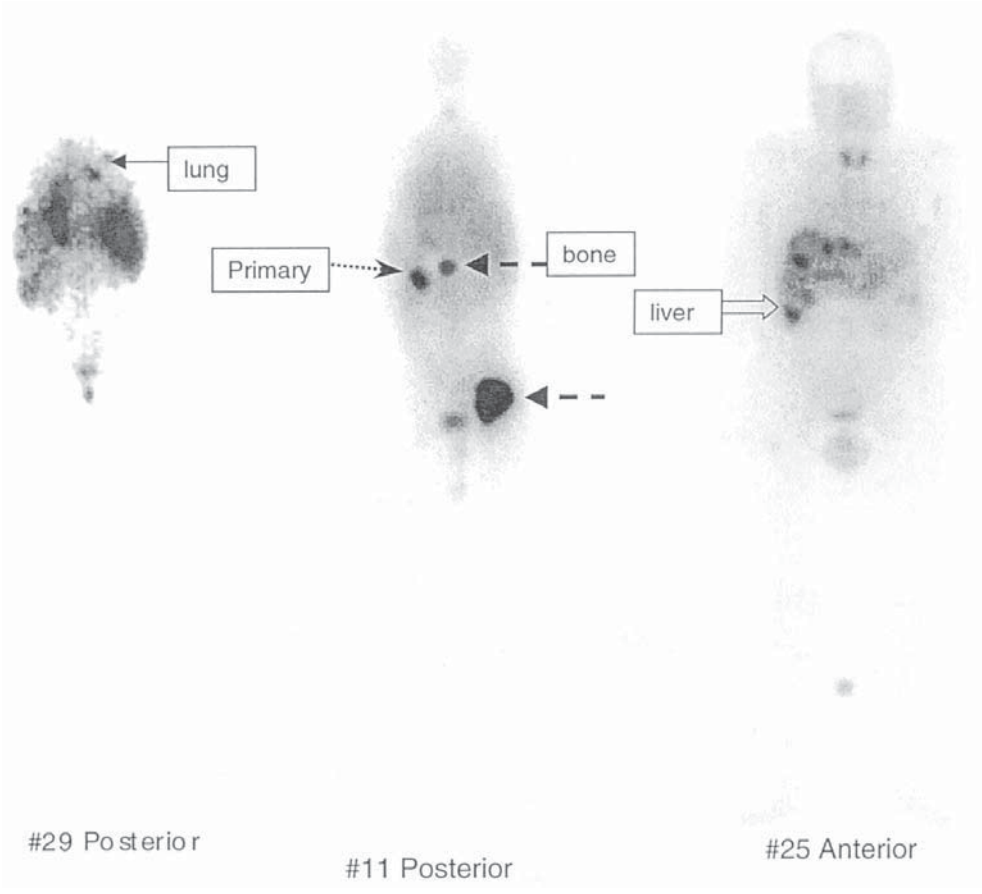

Fig. 2. Typical whole body images recorded during the first week after infusion of three patients who had pulmonary, bone, and liver metastases, respectively. *Arrows* indicate specific visible lesions. Arrow style is consistent among lesion types. *Anterior:* anterior view; *Posterior:* posterior view indicated below the respective scans. # refers to patient number in the trial.

was defined as that dose at which not more than a third of patients had Grade 3 or greater hematopoietic toxicity.

At the very first dose level (30 mCi/m^2 ^{131}I), one of three patients had transient hyperbilirubinemia lasting less than 2 wk. During recruitment into the next dose level (45 mCi/m^2 ^{131}I), a protocol modification was approved by the IRB and the FDA considering hepatic toxicity dose-limiting only when it persisted for two or more weeks. Six patients were entered into this cohort, whereas awaiting approval of the modification. No patient had prolonged hepatic toxicity, and this transient toxicity was therefore not considered dose-limiting. Transient reversible liver function test abnormalities were observed in the majority of patients (27/33). There was no correlation between the amount of ^{131}I administered, or hepatic absorbed radiation dose (median: 0.073 Gy/mCi), and the extent or nature of hepatic toxicity.

Two of the first six patients at 90 mCi/m^2 had ⊕ Grade 3 thrombocytopenia; the MTD was determined to be 90 mCi/m^2 ^{131}I, and a total of 15 patients were studied at this dose level. Hematologic toxicity was correlated with whole body absorbed radiation dose *(24)*. All patients with whole body doses greater than 1.2 Gy experienced a grade 3 or 4 toxic-

ity. Fig. 1 details the platelet counts over time in the first six patients treated at the MTD_A. Severe toxicity did not last more than a week in any patient. No patient required platelet transfusion. Red blood cell counts were unchanged after therapy. Hematopoietic toxicity in this study was comparable to that seen in other studies with ^{131}I-labeled antibodies, confirming that dose-limiting toxicity was radionuclide-dependent.

Because immunohistochemistry was not carried out in all patients, there was targeting of radioactivity to all known tumor sites ⊕2 cm, confirming the high fraction of G250 antigen expressing tumors in patients with the clear cell subtype of RCC. Lesions ⊕ 2 cm in size independent of location were visualized by scintigraphy by the first imaging scan, between 2 and 4 days after administration of ^{131}I-G250. Targeting was comparable to the primary tumor (in those patients who had not had nephrectomy), as well as to bone, liver, lung, nodal, and subcutaneous metastases.

All patients developed human anti-mouse antibodies (HAMA) within 4 wk posttherapy; retreatment was therefore not possible. Seventeen of 33 evaluable patients had stable disease. There were no major responses.

6. CHIMERIZED (HUMAN/MOUSE) VERSION OF G250

In order to allow repeated dosing, a chimerized version of G250 (cG250) has been engineered. In this construct, the murine Fc region has been replaced by a human Fc region. This genetic manipulation eliminates most of the immunogenic murine peptide sequences while maintaining the murine Fab region and, therefore, the specificity of CA IX binding. Initial experience with this construct in a protein dose escalation study in patients indicates that, after a single dose of cG250, two of 16 patients developed a weak human antichimeric antibody (HACA) immune response to the construct measurable at 12 wk postinjection *(25)*. This HACA response was substantially rarer and weaker than seen with the mouse G250 antibody. cG250 was anticipated to, therefore, provide the possibility of repeated doses in patients. cG250 was able to localize to clear cell RCC as well as the parent murine version. Dosimetry analysis indicated that lymph node and bone metastases received approximately 0.20–0.23 Gy/mCi. Assuming a 2Gy maximum tolerated dose to bone marrow (the dose-limiting organ), 200 mCi should be tolerable. This would yield radiation absorbed doses to tumor close to sterilizing levels. Single high-dose radioimmunotherapy with bone marrow support or multiple dose therapy with or without bone marrow support can theoretically achieve major responses. cG250 is now in phase I trials in patients with measurable metastatic RCC. A trial in the Netherlands is studying single, high-dose therapy while a multiple, fractionated low-dose (30 mCi/dose) regimen is being studied in New York. Furthermore, since unconjugated cG250 mediates antibody dependent cellular cytotoxicity, trials of "naked" cG250, with or without cytokines, are in the planning stages.

7. PHASE I PROTEIN DOSE ESCALATION STUDY OF ^{131}I-LABELED cG250

A phase I protein dose escalation study was performed to determine the pharmacokinetics, toxicity, immunogenicity, and imaging characteristics of ^{131}I-labeled chimeric mAb G250 in patients with RCC *(25)*. Chimerization resulted in a major decrease of the immunogenicity of this antibody: no measurable immune responses were detected against the human part of cG250. The minimal, presumably clinically nonrelevant HACA responses,

observed in two out of 16 patients, illustrate the highly reduced immunogenicity of cG250 as compared to its murine progenitor. Therefore, multiple treatments with cG250 seem feasible. These observations are in accordance with the results of other studies: Meredith et al. *(28)* showed that chimerization of mAb 17-1A highly reduced the immunogenicity and Buist et al. *(29)* found similar results with chimeric mAb MOv18.

The in vitro binding characteristics of cG250 were similar to those of mG250, demonstrating that chimerization of the antibody did not affect specificity, affinity, or avidity. In general, the in vivo behavior, including the half-life ($t_{1/2}\beta$), of cG250 was comparable to mG250 ($t_{1/2}\beta$ cG250 68.5 h versus $t_{1/2}\beta$ mG250 47 h). The first chimerized antibodies, e.g., chimeric mAb 17-1A and chimeric mAb B72.3 showed much longer half-lives in patients than their murine progenitors, resulting in relatively poor tumor/nontumor ratios, considered a disadvantage in radioimmunotherapy. However, other chimerized antibodies, e.g., chimeric mAb MOv18, chimeric anti-CEA antibodies and chimeric mAb LL2 have shown half-lives similar to their murine counterpart, as was observed for chimeric mAb G250.

Antigen-mediated tumor uptake of ^{131}I-cG250 was demonstrated by the difference in uptake between antigen-positive versus antigen-negative tumors: uptake in samples of antigen-negative tumors (7 days p.i.) did not exceed 0.0040 %ID/g (blood 7 days p.i. 0.0042 %ID/g), whereas uptake in antigen-positive tumors was as high as 0.5233 %ID/g (blood: 0.0028 %ID/g). Extensive sampling of the primary tumors showed that regional differences in tumor uptake were as high as two orders of magnitude.

Tumor uptake exceeding 0.1%ID/g was observed only at the 2, 5, and 10 mg dose levels, while maximum uptake at the 25 and 50 mg dose level was 0.0170 %ID/g and 0.0120 %ID/g, respectively, suggesting saturation of accessible G250 epitopes in the tumor at the higher protein doses. In the study with mG250 a similar relative decrease in tumor uptake with increasing protein dose was observed. In contrast, in investigations with other antitumor antibodies, doses of 10 mg/kg or more have been administered without any indication of tumor saturation.

Saturable, antigen-mediated liver uptake was observed with cG250, similar to mG250. This liver uptake is in accordance with the known antigen expression on the larger bile ducts, and comparable to mG250 uptake.

All antigen-positive primary tumors as well as all metastatic lesions, as identified by conventional imaging techniques, were visualized. Bander et al. reported 90% successful imaging of primary RCC sites with ^{131}I-mG250 *(27)*. Additionally, occult lesions, confirmed at surgery were visualized. In our study *(25)*, no additional lesions were detected. Larger, prospective studies are needed to evaluate the diagnostic potential of mAb G250 as an imaging agent.

Dosimetric analyses indicated that radiation absorbed doses ranging from 6 to 48 cGy/mCi were delivered to the primary tumors. More importantly, a number of regional lymph node metastases received 20 cGy/mCi and a bone metastasis received 23 cGy/mCi. In solid tumors, responses of radioimmunotherapy can be expected when radiation absorbed doses exceeding 5000 cGy are delivered to the tumor lesions. For bone marrow 200 cGy is considered to be the maximum tolerated radiation dose in radioimmunotherapy. Assuming the bone marrow to be the dose limiting organ, doses as high as 200 mCi ^{131}I-cG250 can be administered safely. Thus, radiation absorbed doses close to tumor-sterilizing levels seem achievable. This study showed that cG250 might be a good candidate for

radioimmunotherapy of RCC. The highly reduced immunogenicity opened the possibility of multiple treatment therapy.

8. PHASE ^{131}I DOSE ESCALATION STUDY OF cG250

Based on the cG250 protein dose escalation study, a ^{131}I-cG250 dose escalation study was performed where patients received a scout dose of 5 mg ^{131}I-cG250 (6 mCi) to define cG250 uptake, followed 1 wk later with 5 mg high dose ^{131}I-cG250, provided the scout dose showed appreciable tumor uptake.

Twelve patients with metastatic RCC were studied. All patients had undergone a prior nephrectomy and had measurable, progressing disease at the time of treatment. After the diagnostic ^{131}I-cG250 injection, metastatic RCC lesions were adequately visualized in nine out of 12 patients. In general, metastatic tumor lesions were visualized from 1 to 2 days p.i. onwards. Because of the background clearance of ^{131}I-cG250, image quality improved with time.

One patient (#4) showed good visualization of metastases, but did not receive a second, high-activity dose of ^{131}I-cG250 administration. This patient developed neurological complaints because of a bone metastasis in the base of the skull, which required external beam irradiation. Afterwards, his clinical condition worsened, not allowing re-entry into the study. Thus, eight patients received a second injection.

The immunoscintigrams obtained after the first injection (7 days p.i.) were almost identical to the immunoscintigrams obtained after the second injection (7, 14, and 21 days p.i.). This confirmed that the distribution of the therapeutic injection can be accurately predicted on the basis of the scans obtained after the administration of the tracer dose. Both injections of the radiolabeled antibody were well-tolerated by all patients and no direct side effects were observed. During hospitalization, most patients complained of mild nausea (without vomiting) and fatigue.

Two to three weeks after the high-dose ^{131}I-cG250 injection, a drop in platelet and leukocyte counts was observed in all patients, with a nadir between 4 and 6 wk. At the 1665 MBq/m^2 dose level, grade II hematological toxicity was observed in one patient (thrombocytopenia and leukocytopenia). At the 2220 MBq/m^2 dose level, one patient showed grade II thrombocytopenia and leukocytopenia, whereas another patient showed grade II thrombocytopenia and grade III leukocytopenia. Both patients, treated at 2775 MBq/m^2, showed grade IV hematological toxicity (thrombocytopenia and leukocytopenia) and required two platelet infusions. Nonhematological toxicity did not exceed grade I (nausea without vomiting, fatigue without decrease in daily activities). Based on the observations in these eight patients, MTD has been set at 2220 MBq/m^2.

A remarkable difference between radioimmunotherapy with chimeric mAb cG250 and murine mAb G250 is the absence of any nonhematological toxicity. In the previous radioimmunotherapy trial with murine mAb G250, all patients showed transient hepatic dysfunction, which in some cases led to transient icterus *(26)*. In contrast to the trial with murine mAb G250, patients in the cG250 trial received a diagnostic dose of mAb, prior to administration of high-dose ^{131}I-cG250. The absence of liver toxicity may be explained by saturation of the hepatic compartment by the first dose *(25,26)*. Another possible explanation may be the higher hepatic uptake of murine mAb G250 compared to chimeric mAb cG250. At equal doses, liver uptake of murine mAb G250 *(26)* was 2–3 times higher than the liver uptake of chimeric mAb cG250 *(25)*.

Another difference between the murine ^{131}I-G250 and the chimeric ^{131}I-cG250 is the lower MTD of ^{131}I-cG250. The hematologic toxicity encountered with murine ^{131}I-G250 indicated an MTD of 3330 MBq/m^2 *(24)*, whereas MTD in this study was 2220 MBq/m^2. The lower MTD of chimeric ^{131}I-cG250 can be explained by the longer circulation time of chimeric mAb cG250 compared to murine mAb G250 ($t_{1/2}\beta$: 69 h vs 47 h, respectively). The longer circulation time resulted in increased whole body radiation leading to the earlier encountered hematologic toxicity.

Two out of eight patients who received a therapeutic injection of ^{131}I-cG250 showed an antitumor response whereas the other six patients showed progression of disease. Both patients that showed a response were treated at the 2220 MBq/m^2 dose level. In the first patient (#5), stable disease was achieved, lasting 3–6 months. In the second patient (#8), a partial response (>50 % reduction in size of tumor lesions) was observed which is ongoing (>9 months).

In one patient (#9), a positive HACA response was detected in the serum sample obtained prior to the first ^{131}I-cG250 administration as well as in all subsequent samples. This patient showed more rapid whole body clearance as determined by the radioimmunoscintigrams. Four months prior to participation in the current trial, this patient had participated in another clinical study in which RCC patients with a primary tumor had received two injections of 5 mg mAb cG250 given 4 days apart. At that time, the patient showed positive uptake of mAb cG250 in his primary tumor. No HACA responses were detected in the sera of any other patients collected up to 10 wk after the second ^{131}I-cG250 administration.

As demonstrated by the immunoscintigrams after the second injection, mAb cG250 allows for delivery of radiation to RCC tumor lesions for at least 3 wk p.i. The dosimetric analyses of the radioimmunoscintigrams corresponded better with the observed toxicity compared to the administered antibody dose per body surface area. These findings are in accordance with the results of other investigators. Sgouros et al. *(30)* reported that determination of the whole body radiation absorbed dose was the best predictor for bone marrow toxicity.

One partial response and one stabilization of disease was observed in RCC patients with documented progressive disease prior to study entry. These results warrant further study in a phase II setting at MTD. cG250 is a very suitable candidate for radioimmunotherapy of RCC. Several strategies—necessary to increase the radiation dose to tumors—are available and future studies will focus on optimization of this therapeutic approach for patients with metastasized RCC.

9. HETEROGENOUS cG250 mAb UPTAKE IN TUMORS: EFFECT OF MULTIPLE INJECTIONS

As mentioned earlier, tumor uptake of chimeric monoclonal antibody (mAb) G250 (cG250) in patients with primary RCC is amongst the highest reported in solid tumors. However, as observed in other tumor types, the intratumoral distribution of the antibody is highly heterogeneous: in some cases, regional differences in intratumoral mAb cG250 uptake exceed a factor 100. This may limit the efficacy of radioimmunotherapy. In a subsequent study, we showed that this heterogeneous mAb cG250 distribution could not be attributed solely to 1. antigen expression, 2. blood vessel density, 3. reactive stromal tissue, or 4. necrosis.

To further analyze the heterogeneous mAb cG250 tumor uptake, a study was performed to investigate whether the tumor uptake is influenced by dynamic factors, e.g., heterogeneous blood supply, elevated interstitial pressure, or large transport distances in the interstitium. If these factors play a major role in the intratumoral distribution of antibodies, the mAb cG250 distribution over an RCC tumor is likely to change with time. That is, differences in the distribution of two consecutive injections would be expected or, in other words, repetitive injections would target different areas within a tumor.

Ten patients with a clinical diagnosis of primary RCC were studied. Nine days before surgery patients received [^{125}I]-cG250 (5 mg cG250, 50 μCi ^{125}I) followed by a second injection of [^{131}I]-cG250 (5 mg cG250, 3.5 mCi ^{131}I) four days later. Postsurgery, a tumor slice was mapped and cut into 1 cm^3 cubes. Each cube was analyzed for [^{125}I]-cG250 and [^{131}I]-cG250 uptake and the ^{131}I/^{125}I-ratio was determined. For each tumor slice, the distribution patterns of both isotopes were reconstructed and compared to each other.

All tumors analyzed showed a heterogeneous distribution of both isotopes throughout the tumor slice; focal uptake in some areas of a tumor reached very high levels (up to 0.19% ID/g) whereas other tumorous areas of the same slice showed much lower uptake (as low as 0.0047% ID/g). Surprisingly, the distribution pattern of both injections was identical in all tumors examined: without any exception, in all samples analyzed ($n = 692$) the uptake of [^{125}I]-cG250 was similar to [^{131}I]-cG250 uptake. In none of the investigated tumor samples ($n = 692$) did the differences between [^{125}I]-cG250 and [^{131}I]-cG250 uptake exceed a factor of 3, whereas regional differences (i.e., different samples) in uptake of the same antibody injection sometimes exceeded a factor of 40 or more. The strikingly similar three-dimensionally displayed distribution patterns of both injections over a tumor nicely illustrate this identical behavior. This is an unexpected finding in view of the temporal and spatial blood flow heterogeneity. Overall, the ^{131}I/^{125}I-ratio was 1.72 ± 0.45 (mean ± S.D.). The constant ^{131}I/^{125}I-ratios, observed in all tumor samples investigated, indicate that the tumor parameters governing mAb cG250 uptake do not alter significantly within the time period studied. This observation might have significant impact on fractionated radioimmunotherapy: the separate fractions must be sufficiently spaced to allow targeting of different tumor areas, possibly accessible by radiation-induced alterations in the tumor. Additionally, heterogeneous tumor uptake may be less prominent in smaller tumors, circumventing insufficient cG250 uptake in particular areas.

10. FRACTIONATED RADIOIMMUNOTHERAPY WITH MULTIPLE INJECTIONS OF ^{131}I-LABELED CG250

Experimental data suggests that multiple administrations of radiolabeled antibody may have greater therapeutic effect than a single infusion *(30,31)*. A Phase I/II radioimmunotherapy trial is, therefore, under way at Memorial Sloan-Kettering Cancer Center to evaluate the utility of multiple, outpatient, administrations of ^{131}I-cG250 in patients with measurable metastatic RCC. The lack of immunogenicity permitted a design whereby whole body and serum clearance characteristics of an initial dose of ^{131}I-cG250 are used, using a two-compartment model, to calculate fractionated radioimmunotherapy with multiple doses of ^{131}I-cG250 that would deliver a specified whole body radiation dose. Starting with an initial dose of 30 mCi/5 mg ^{131}I-cG250, varying amounts of ^{131}I-cG250 are administered at 2–3 day intervals such that the total amount of radioactivity in the body does not exceed 30 mCi ^{131}I. Retreatment is given at intervals of between 8 and 12 wk if there is no evidence of disease progression.

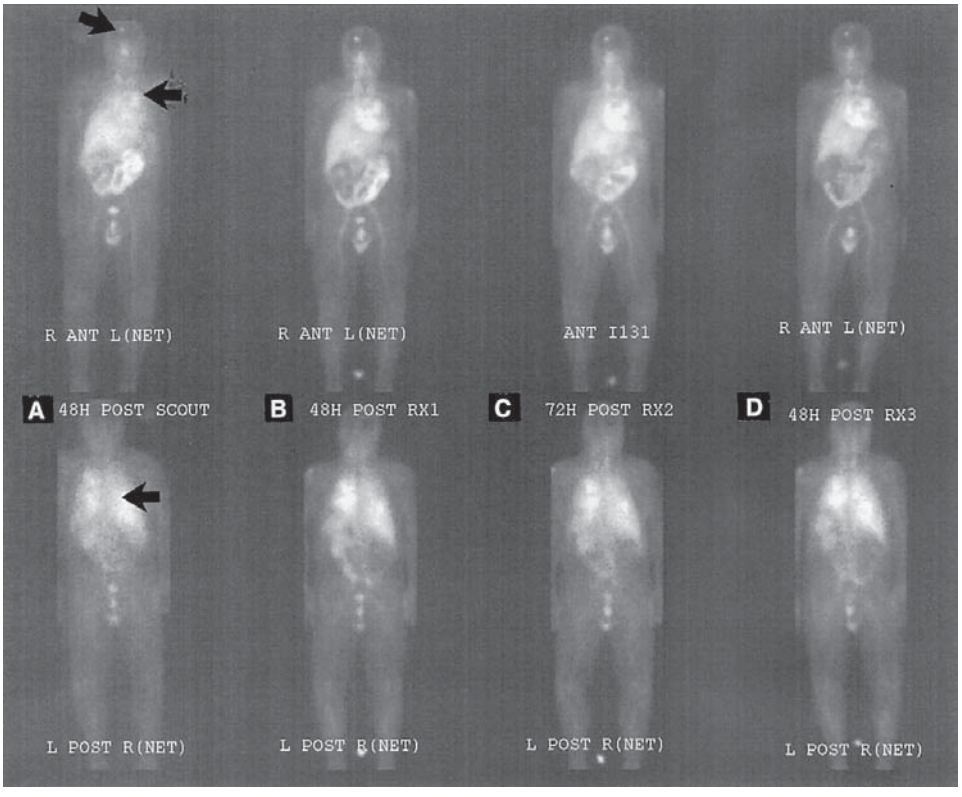

Fig. 3. Serial images (anterior, top; posterior, bottom) after ^{131}I chimeric G250. (**A**) After scout ^{131}I-cG250; (**B**), (**C**), and (**D**) 48–72 h after therapeutic cG250. Note the comparable kinetics, and targeting of antibody to the frontal subcutaneous lesion, the hilar, and lung lesions (arrows).

Three patients were treated at a whole body radiation dose of 50 cGy, without severe toxicity. One of five evaluable patients at the 75 cGy whole body radiation dose developed Grade 3 hematopoietic toxicity. In contrast to the radioimmunotherapy trial with ^{131}I-murine G250, there has been no hepatic toxicity, presumably because of saturation of hepatic receptors by the scout dose of cG250. Moreover, an antiantibody response, manifest by altered serum and whole body clearance of radioactivity, and by detection of serum human antichimeric antibody (HACA), has been seen in only one of the eight patients treated thus far. Two patients have received two treatment courses (five and eight infusions each, respectively) and one patient has received three treatment courses (five infusions each). There has been close correlation between actual and predicted clearance of all treatments, except for the second course of treatment in the one patient who developed HACA. Targeting to tumor has been excellent in all patients, and targeting of each treatment has been comparable. Fig. 3 shows whole body images obtained at comparable time points in a patient treated at the 50 cGy whole body dose. There have been no major responses so far; dose escalation continues.

In summary, there is strong indirect evidence that the host immune system is intimately involved in the natural history of renal cancer. Immunological studies are providing information on tumor-related differentiation antigens of the kidney and beginning to allow molecular subclassification of renal cancer subtypes. Initial indications are that

these subtypes have practical clinical relevance. Last, mAb probes, which have clearly proven their value as in vitro diagnostics, are now demonstrating a role as in vivo imaging agents and therapeutics in nonneoplastic and neoplastic diseases. Efforts continue to develop such an approach in RCC.

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23 Adoptive Immunotherapy in Renal Cell Carcinoma

Barbara J. Gitlitz, Arie S. Belldegrun, and Robert A. Figlin

Contents

1. INTRODUCTION

The study of cancer immunotherapy has gained increasing popularity since Dr. William Coleys' observations in the late 19th century of patient tumor shrinkage after life-threatening bacterial infections. The idea that tumor cells, similar to invading foreign pathogens, can express abnormal antigens has formed the basis of attempts to manipulate the immune system to cause improved tumor surveillance and destruction. Over the last two decades, considerable strides have been made in the field of tumor immunology. This includes further knowledge of antigen processing and presentation via the major histocompatability complex *(1,2)*; understanding the interaction between T cells and antigen presenting cells (APC) via the T-cell receptor (TCR) *(3)*, secondary signals such as costimulatory molecules, regulation of the immune response by cytokines, and most notably the characterization of a variety of genes-encoding tumor-associated antigens (TAA). For many tumor types, the DNA and amino acid sequences of TAA have been worked out including the immunodominant peptide restricted by major histocompatibility complex (MHC) rules. These relatively recent discoveries have allowed for the exploration of more targeted immunotherapies. The transfer of cells with antitumor reactivity is termed adoptive immunotherapy and shows promise in the treatment of selected malignancies. Adoptively transferred T cells have been shown to bring about regression in

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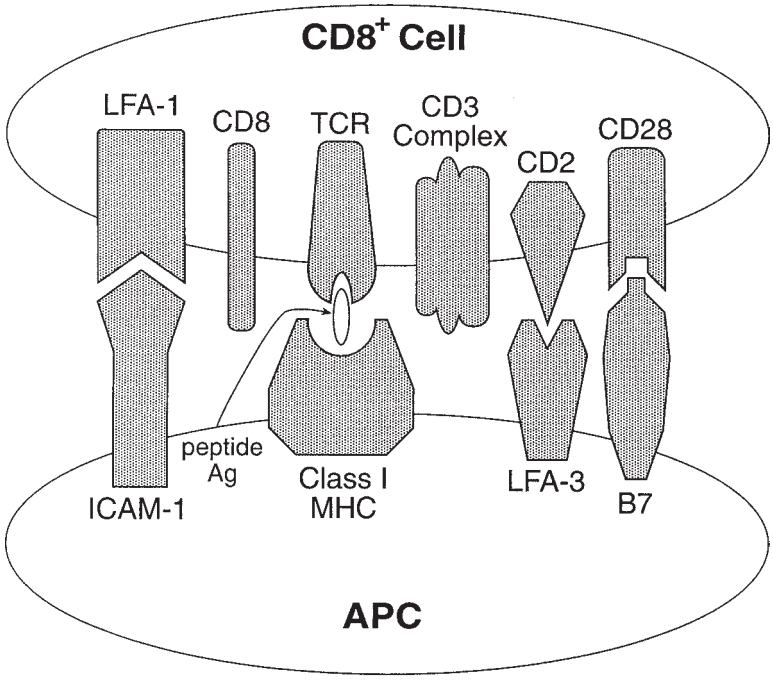

Fig. 1. Antigen presentation to $CD4^+$ and $CD8^+$ lymphocytes.

animal tumor models *(4–6)*, and have been studied in human clinical trials since the 1980's. It is now evident that the potential of adoptive therapy seen in animal models has not yet fully translated into human models. However, there are well-documented studies demonstrating durable responses in human cancers, most notably renal cell carcinoma (RCC) and melanoma. This chapter will focus on the development of adoptive immunotherapy for RCC, its shortcomings, and potential for improvement. Adoptive immunotherapy employing autolymphocyte therapy (ALT), lymphokine-activated killer cells (LAK), and tumor infiltrating lymphocytes (TIL) will be featured, as will other promising approaches.

2. CELL-MEDIATED IMMUNE RESPONSE

The cellular arm of the immune system is central to the rejection of tumors. Antigen, both normal and foreign, is continuously presented by APC via MHC class I and class II antigen presentation *(1,2)*. In humans, the MHC is the human lymphocyte antigen (HLA) gene cluster located on chromosome 6 and encodes for class I and class II molecules *(7)*.

T lymphocytes are the effectors of the cellular immune system and recognize antigen via the TCR. The TCR is formed by recombination of germline genes enabling a wide diversity of receptors. In all mature human T cells, the TCR is associated with a glycoprotein complex called CD3. There are two major T-cell subsets based on TCR-restricted recognition of class I or class II molecules. It appears that the TCR binds to a small antigenic peptide located in a grove of the MHC molecule *(3)*. The $CD8^+$ subset of T cells shows affinity for recognition of antigen in association with class I molecules *(8)*. The $CD4^+$ subset of T cells show affinity for recognition of antigen in association with class

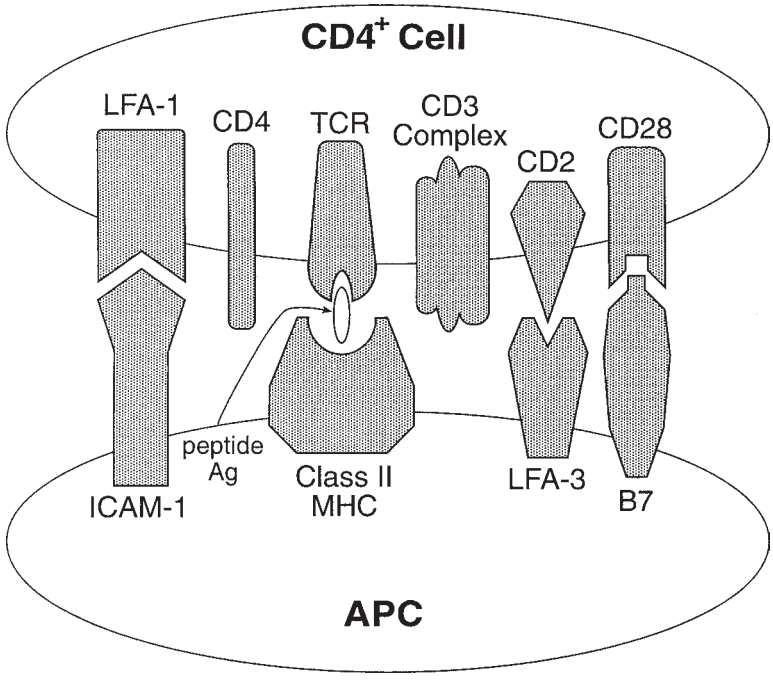

Fig. 2. Antigen presentation to $CD4^+$ and $CD8^+$ lymphocytes.

II molecules *(9)* (Figs. 1 and 2). The binding of the TCR to the MHC-antigen complex is crucial to the generation of effector cell function, including target cell lysis, clonal cell expansion, and secretion of cytokines.

Cytokines are proteins produced by mononuclear cells of the immune system and, like true hormones, have regulatory actions on other immune cells at a distance from the secreting cell. The discovery of the cytokine interleukin-2 (IL-2) has had an enormous impact on cancer immunology. In 1976, Morgan et al. demonstrated a soluble factor in the medium of cultured PHA-stimulated lymphocytes that supported long-term cultures of bone marrow-derived cell suspensions *(10)*. The cells proliferating in response to this growth factor were indeed, characterized as T lymphocytes *(11)*. Both $CD4^+$ and $CD8^+$ T cells could be expanded and maintain their in vivo and in vitro activity in the presence of this growth factor, later to be known as IL-2.

IL-2 is manufactured predominantly by activated T cells. When a mature T cell encounters its specific antigen-MHC complex, signals transduced across the plasma membrane result in the transcriptional activation of the IL-2 gene and genes encoding for the IL-2 receptor *(12)*. Ligation of IL-2 to its membrane receptor results in cell cycle progression of the activated T lymphocyte and subsequent antigen-specific T-cell clonal expansion *(13)*. The cytotoxic activity of $CD8^+$ T cells and monocytes is stimulated *(14)*. IL-2-stimulated monocytes elaborate other cytokines, such as interferons (IFNs) and tumor necrosis factor (TNF), which are additive to the immune effect *(15)*. IL-2 has other activating and some inhibitory effects on the immune response, with a net stimulatory function.

The antineoplastic effect of IL-2 has been documented in tumor-bearing animal models, causing regression of established metastases from selected murine tumors *(16)*. Most of the clinical studies of IL-2 have been in patients with advanced RCCa and melanoma.

Numerous trials have confirmed the efficacy of IL-2 in human RCC *(17,18)*. Based on data from seven clinical trials involving a total of 255 patients, the U.S. Food and Drug Administration approved the use of IL-2 for the treatment of mRCC in 1992.

The availability of IL-2 has allowed for major advances in the understanding of rejection of human cancers using immune cells. The discovery of IL-2 was the key, allowing for in vitro expansion of lymphocyte clones responsive to antigen in vivo. Enhanced therapeutic efficacy was observed when cultured immune cells used in adoptive transfer were accompanied by exogenous IL-2 administration *(19,20)*. This exogenous use of IL-2 was also shown to cause in vivo proliferation and prolonged survival of cells used for adoptive immunotherapy *(21)*. Based on these observations, exogenous IL-2 is often administered with the adoptive transfer of cultured immune effector cells.

3. AUTOLYMPHOCYTE CELLULAR THERAPY

ALT is adoptive cellular therapy of neoplastic disease using ex vivo activation of autologous lymphocytes from tumor bearing hosts using low doses of anti-CD3 monoclonal antibody (mAb) and a mixture of previously prepared autologous cytokines (T3CS). Triggering the CD3 component of the TCR results in clonal T-cell proliferation through an IL-2-dependent autocrine pathway *(22)*. The theoretical basis of ALT relies on the activation of memory T lymphocytes by T3CS in the tumor bearing host. These T lympho-cytes presumably have been exposed in vivo to tumor antigens and may possess the potential for mediating tumor regression following nonspecific activation. Preclinical murine studies involved the adoptive infusion of donor memory cells (splenocytes) prepared ex vivo using and anti-CD3 mAb and T3CS *(23,24)*. Expansion of $CD44^+$ (memory T cells) were the principal mediators of antitumor effects and protection from subsequent tumor challenge.

The preparation of ALT for immunotherapy is a multistep process. First, patients undergo pheresis, and approximately $2 \leftrightarrow 10^9$ (PBL) are harvested. The cells are incubated for 3 d in the presence of anti-CD3 mAb, and the supernatant fluid, called T3CS, is collected and frozen at –80°C. Biochemical analysis of the T3CS has revealed the presence of various cytokines, including interleukin-1-alpha (IL-1-α), IL-1-β, IL-6, interferon-gamma (IFN-γ), TNF-β, GM-CSF, and both the soluble IL-2 receptor and anti-CD3 mAb *(25)*.

Two weeks after the initial pheresis, patients undergo repeat pheresis for the collection of PBL (presumably containing memory T cells) for activation. The cells are incubated for 5 d at 39°C in media containing 25% T3CS, indomethacin, and cimetidine. The theoretical basis for the use of cimetidine relies on the observation that some suppressor T-lymphocyte subpopulations contain H2 receptors, and the blockade of these receptors during activation with CD3 may prevent the expansion of suppressor T-cell clones *(26)*. The basis for the use of indomethacin is the observation that prostaglandin E may inhibit IL-2-mediated T-cell proliferation, and the use of nonsteroidal antiinflammatory agents may block this inhibition *(27)*. Following the 5-d incubation, activated cells are irradiated to 50 cGy to reduce the activity of suppressor T lymphocytes *(28)*, and the cells are then infused into patients. Patients continue to receive oral high-dose cimetidine during therapy, and therapy is given monthly over 6 months.

In 1990, an initial report of a 90-patient randomized trial of ALT versus high-dose cimetidine alone for the treatment of metastatic RCCa was published *(29)*. Six doses of 10^9 ALT were given on a monthly basis as an outpatient infusion, without dose limiting

toxicity. Statistically significant findings included a 2.5-fold survival advantage in patients receiving ALT (21 months versus 8.5 months) over cimetidine alone. In addition, those patients with >500 pg of IL-1 in their T3CS had a sixfold survival advantage. Unexpected findings in the report included an improved response rate in patients receiving ALT, (21%), but a lack of correlation between response and survival; and males who received ALT had a fourfold survival advantage, whereas females receiving ALT demonstrated no survival advantage.

This initial report has been updated *(30,31)*, and over 300 patients with mRCC have been accrued into this multiinstitutional clinical trial, with a persistent survival advantage being reported in the ALT arm. The early success of ALT in the treatment of mRCC led to the establishment of a number of proprietary ALT treatment centers. A randomized trial comparing ALT to single agent IL-2 therapy, the only FDA approved therapy for mRCC, would be a valid test of the ability of ALT to improve survival in patients with mRCC. ALT, is currently being studied in a large-scale phase III trial comparing ALT to single agent IFN-α.

There has been a small randomized trial of ALT vs observation for the adjuvant treatment of RCC *(32)*. Forty-five patients were randomized according to stage, gender, time from nephrectomy, and serum IL-1 level. They found a significant difference in favor of ALT over observation for overall median time to progression. In further subgroup analysis, there was an advantage in median time to recurrence in patients with node positive disease, and T3 stage. This founded the basis for larger confirmatory trials.

4. LYMPHOKINE ACTIVATED KILLER CELLS

LAK cells are peripherally circulating lymphoid cells activated in vitro by the exposure to pharmacologically high concentrations of IL-2 (usually 500 to 1000 IU/mL).

In the original description of the LAK phenomenon by Grimm et al. *(33,34)*, lymphokine activation of PBL from cancer patients or normal individuals caused the expression of cytotoxicity toward a variety of natural killer cell (NK) resistant, autologous and allogeneic solid tumors. In contrast to the MHC-restricted cytotoxicity of cytotoxic T lymphocytes (CTL); lymphokine-activated killer (LAK) activity is MHC nonrestricted, lysing tumor target cells derived from syngeneic, allogeneic, or xenogeneic sources regardless of target expression of MHC antigens.

When generated from human PBL, LAK precursors are contained primarily in the large granular lymphocyte (LGL) population containing virtually all active NK cells *(35)*. These precursors express surface markers characteristic of NK cells, including $CD56^+$ (Leu-19) and $CD16^+$ (Leu-11), and rarely express the T-cell-associated markers CD3 or CD5 *(36)*. DM1 is another surface marker reported to identify the majority of human LAK precursors *(37)*.

The capacity to distinguish between tumor cells and normal cells is a hallmark of LAK activity, although the antigen receptors expressed on cells mediating MHC-nonrestricted killing are largely unknown. Once target cell adhesion takes place, a number of calcium-dependent phases of LAK cell lytic function occur. These include granule reorientation, granule exocytosis, and perforation of target cell membrane by granule-associated pore-forming proteins *(38)*. In addition to cytotoxic granule release, LAK cells express a membrane-associated toxin called M-CTX, which also causes lytic activity against nucleated tumor targets *(39)*.

Murine models with experimentally induced metastases demonstrated that the passive transfer of LAK plus IL-2 caused the regression of established lung, liver, or subcutaneous metastases in mice bearing tumors from a variety of cancer types *(4,40,41)*. In these animal models, the antitumor effect of LAK cells was dependent on the number of cells administered, the dose of IL-2, and the size of the tumor burden. In most studies, the combined administration of LAK cells plus IL-2 led to improved efficacy over IL-2 alone. These animal studies also demonstrated the trophic effect of IL-2, causing in vivo expansion and proliferation of LAK cells and LAK cell death when discontinued *(5)*.

Human phase I studies were undertaken evaluating the safety of adoptively transferred LAK cells plus IL-2 in patients with advanced cancers who failed standard therapy. Initial studies using activated killer cells alone showed no clinical efficacy, but demonstrated the tolerability of multiple infusions of up to 2 ↔ 10^{11} cells with minimal side effects *(42,43)*. In the first reported trial combining LAK plus IL-2, 11 of 25 patients experienced objective tumor response (1 CR and 10 PR) *(44)*. These responses occurred in patients with four histologic tumor types: renal cell, melanoma, lung, and colon carcinoma. Tumor responses were seen in lung, liver, and subcutaneous tissues. This landmark study demonstrated the feasibility of adoptive immunotherapy of human cancers.

To generate LAK cells, patients are initially treated with IL-2 and undergo leukopheresis 48 to 72 h following the discontinuation of IL-2. Treatment with IL-2 induces lymphopenia, followed by rebound lymphocytosis, with leukopheresis performed at the peak of the rebound lymphocytosis. Both low-dose (2 to 12 ↔ 10^6 IU/kg) and high-dose (6 to 7.2 ↔ 10^5 IU/kg) IL-2 *(45,46)* have been used. The PBL are then cultured in vitro in high concentrations of IL-2 (400 to 1000 IU/mL), and approximately 10^{10} to 10^{11} LAK cells are generated and then reinfused. The methods of concomitant IL-2 administration have included high-dose regimens (6 to 7.2 ↔ 10^5 IU/kg) administered by intravenous bolus infusion every 8 h for 4–5 d or lower dose regimens (1 to 6 ↔ 10^6 IU/m^2/d) administered by continuous intravenous infusion over four or more days. The toxicities of concomitant therapy with LAK cells plus IL-2 are related to the dose of IL-2 used. The optimal dose and schedule of IL-2 administration with LAK cells are not defined. Studies have found less clinical toxicity with lower-dose continuous intravenous IL-2 infusion regimens when compared with bolus IL-2 infusions *(47)*. Moreover, some studies have found an improved response rate using continuous infusion schedules *(48)*. A randomized phase II trial by the NCI-Extramural IL-2 LAK Working Group found equivalent anticancer activity and toxicity using high-dose IL-2 as a bolus versus high dose continuous infusion IL-2 in the treatment of patients with mRCC *(49)*.

Early studies using IL-2 plus LAK cells in 180 consecutively treated cancer patients showed responses predominantly in patients with mRCC or metastatic melanoma. Of 72 assessable patients with mRCC, a CR was seen in eight patients and a PR in 17 patients, for an overall response rate of 35% *(50)*. Based on these promising results, various institutions conducted phase II studies using LAK cells plus IL-2 in the treatment of mRCC. A summary of the results of these trials is depicted in Table 1 *(49–56)*. These trials used a variety of preparative and treatment regimens, and response rates in mRCC ranged from 9 to 35%, with a combined objective response rate of 23% in 502 patients.

The goal of combined therapy with LAK cells plus IL-2 is to improve on the clinical response rates of IL-2 when used as a single agent. There have been three randomized trials comparing LAK/IL-2 to IL-2 alone. The first was performed by investigators of the Modified Group C Program, entering a total of 167 patients from 13 institutions, includ-

Table 1
Phase II Trials of Combination IL-2/LAK in the Treatment of Metastatic Renal Cell Carcinoma

Author	*No. of Patients*	*Objective Response No.(%)*
Weiss et al. *(49)*	94	16 (17)
Rosenberg et al. *(50)*	72	25 (35)
Parkinson et al. *(51)*	47	4 (9)
Dillman et al. *(52)*	50	7 (14)
Palmer et al. *(53)*	102	17 (18)
Foon et al. *(54)*	23	6 (26)
Thompson et al. *(55)*	42	14 (33)
Gramata et al. *(56)*	72	23 (32)
Total	502	112 (22)

ing 69 patients with mRCC *(57)*. In mRCC patients, the response rates for LAK/IL-2 and IL-2 were 13 and 8%, respectively. These results suggested that LAK cells did not contribute to higher response rates over IL-2 alone. The Surgery Branch of the NCI conducted a prospective randomized trial of 181 patients with advanced cancer, including 96 patients with mRCC *(46)*. The IL-2 regimen for both the induction of lymphocytosis for LAK cell harvest and the treatment arm for all patients consisted of high-dose IL-2 at 7.2 ↔ 10^5 IU/kg administered as an intravenous bolus every 8 h for 4–5 d. There was no statistical difference in response rates (33% LAK plus IL-2 vs 24% IL-2 alone) or in survival (48 month survival: 29% LAK plus IL-2 versus 25% IL-2 alone). In the third trial, performed by investigators sponsored by Hoffman–LaRoche *(58)*, 49 patients were randomized to receive Roche IL-2 (3 ↔ 10^6 U/m^2/d on days 1–5, 13–17, 21–24, and 28–32) with or without LAK cells reinfused on days 13–15. There were two responses (1 CR, 1 PR) in 21 patients randomized to IL-2 plus LAK and three responses (1 CR, 1 PR) in 28 patients receiving IL-2 alone. Although the power of each individual study is relatively low, the combined studies strongly suggest that the combination of LAK cells plus IL-2 has not demonstrated superiority over therapy with IL-2 alone in the treatment of mRCC.

5. TUMOR INFILTRATING LYMPHOCYTES

In 1980, NCI investigators described a technique to isolate and expand infiltrating lymphoid cells from solid tumors in large numbers by using IL-2 *(59)*. Lymphocytes comprise only a small proportion of cells in a neoplastic nodule, some of which contain IL-2 receptors, presumably because of interactions with tumor antigens. Under the influence of IL-2, these lymphocytes can grow in single-cell suspensions of tumor and appear to mediate the destruction of tumor cells, leaving relatively pure cultures of infiltrating lymphocytes. These T cells grown from murine tumors exhibit significant cytotoxicity for syngeneic tumor cells. Subsequent murine studies compared the adoptive transfer of TIL versus LAK cells (both with accompanying IL-2) demonstrating TIL to be 50–100 times more potent on a per cell basis than LAK cells in the treatment of 3-d established lung micrometastases *(60)*. In addition, TIL can eliminate murine pulmonary metastases in the absence of IL-2 administration, although low doses of IL-2 enhance their effectiveness by two- to fivefold *(6)*. In murine models, the combination of TIL, cyclophosphamide,

and IL-2-mediated the cure of 100% of mice with advanced hepatic macrometastases and 50% of mice with pulmonary macrometastases *(60)*. The combination of LAK/IL-2 with or without cyclophosphamide had little effect on these large tumor burdens. It was postulated that cyclophosphamide acted via destruction of suppressor cells and other suppressor factors in transferred immune cells. Mice cured of these tumors using combined treatment were immune to subsequent challenge with the same tumor.

The specificity of TIL antitumor activity is presumably mediated through tumor antigen-TCR interaction and is thus MHC restricted. TIL secrete cytokines, such as GM-CSF, IFN-γ, and TNF-α in response to autologous tumor stimulation. This provides further evidence for immune recognition of tumor antigen *(61)*. IL-2 expanded TIL derived from melanoma and ovarian cancer patients have been shown to contain CTL that are primarily $CD8^+$, recognize tumor cells via the TCR, and are MHC class I restricted *(62,63)*. Unlike the case in melanoma, autologous tumor-specific cytotoxicity has been difficult to demonstrate in RCC. There have been reports, however, identifying CTL with specificity for autologous human RCC *(64,65,66)*. Phenotypically, these TIL are mainly $CD3^+$ $CD8^+$ T cells that are grown in the presence of low-dose IL-2 (20 U/mL) and irradiated autologous tumor stimulation *(65)*. The autologous cytotoxicity is inhibited by anti-CD3 antibody and anticlass I MHC, suggesting that recognition of tumor is via the TCR/CD3 complex. Although the identification of specificity between TIL and tumor antigen in human RCC models has been elusive, experiments have demonstrated HLA-A2 restricted tumor-specific cytotoxicity by a $CD8^+$ CTL line isolated from an uncloned TIL population of primary RCC *(67)*. Other studies have demonstrated restricted TCR V-β and J-β use in ex vivo IL-2 expanded TIL obtained from patients with RCC, suggesting the plausibility of a specific interaction between TIL cell TCR and tumor antigen *(68)*.

Numerous investigators have successfully established TIL cultures from hundreds of different human tumors, including RCC, melanoma, colon and breast cancer, lymphoma, and other tumor types *(64)*. These human TIL have shown in vitro antitumor reactivity *(61)*.

The preparation of TIL for human adoptive cellular immunotherapy in the treatment of mRCC occurs as follows *(69)*. Under aseptic conditions, radical nephrectomy specimens containing the primary tumor are first mechanically and subsequently enzymatically digested (collagenase, hyaluronidase, DNAse) to obtain single-cell suspensions containing both viable mononuclear cells and tumor cells. These cells are expanded ex vivo under sterile conditions in the presence of IL-2. After approximately 2 wk in culture, there is an absence of tumor cells, whereas TIL continue to proliferate. Following 4–6 wk in culture, 10^8 to 10^9 initial mononuclear cells proliferate to approximately 10^{11} TIL, which are infused into patients together with IL-2.

There have been several clinical trials using TIL in the adoptive immunotherapy of advanced human cancers. A pilot trial from the NCI used TIL with varying doses of IL-2, with and without cyclophosphamide, to treat 12 patients *(70)*. Two PRs were observed among the 12 patients, occurring in a patient with metastatic melanoma and another with mRCC. Both responders were among the groups that received cyclophosphamide, IL-2, and TIL doses above the study median. The two responders were also among the group of nine patients whose TIL showed in vitro autologous tumor killing. This study established the feasibility of using TIL in combination therapy of human cancer. Another small study using TIL and low-dose IL-2 without cyclophosphamide in patients with advanced cancer reported an objective response rate in 18% of 28 evaluable patients *(71)*. Responses were again seen in patients with metastatic melanoma and mRCC, with responses lasting

Table 2
Phase I-II Trials of Combination IL-2/TIL in the Treatment of Metastatic Renal Cell Carcinoma

Author	*No. of Patients*	*Objective Response No. (%)*
Dillman et al. *(52)*	6	0
Topalian et al. *(70)*	4	1 (25)
Kradin et al. *(71)*	7	2 (29)
Bukowski et al. *(72,73)*	34	4 (12)
Figlin et al. *(74)*	55	19 (35)
Goedegebuure et al. *(75)*	8	0
	114	26 (23)

from 3 to 14 months. The response rate was similar to that seen in the NCI study but with low-dose IL-2 and without the use of cyclophosphamide.

In comparison to the number of patients treated with LAK/IL-2, few patients with mRCC have received therapy with TIL. Table 2 shows the results of these trials *(52, 70–75)*. The two larger studies (Bukowski et al., Cleveland Clinic *[72, 73]*, Figlin et al., UCLA *[74]*) demonstrate differing response rates (12% in 34 patients versus 35% in 55 patients). This disparity may be explained by significant differences in protocol design. At the Cleveland Clinic, patients were treated in two clinical trials. In the first trial *(72)*, 18 patients were treated with TIL isolated from primary tumor and metastatic sites. Patients were treated with one of four IL-2 dose levels, including level I, in which patients received no IL-2. Patients received a total of 2–4 d infusions of IL-2, and four patients received cyclophosphamide. The response rate for this trial was 0% (0 of 18). In the second trial *(73)*, 16 patients were treated with TIL obtained from primary tumor and expanded in IL-2 and IL-4. The response rate for this trial was 25% (4 of 16). At UCLA, patients received TIL isolated from primary tumor only. All patients received 3 or 4 wk IL-2 infusions using a single low-dose IL-2 regimen with demonstrable activity in the treatment of mRCC *(76)*, and patients received no immunosuppressive agents. The results obtained at UCLA can be summarized as follows *(74)*. Sixty-two patients with mRCC presenting with their primary tumor in place underwent nephrectomy. Following radical nephrectomy, seven patients did not receive further therapy, including five who no longer met protocol requirements to safely receive systemic IL-2, one patient with no growth of TIL, and one patient was found to have transitional cell carcinoma. Thirty-two of the patients received biologically active doses of cytokine prior to radical nephrectomy (IFN-α: 15 patients, TNF-α: 4 patients, IL-2: 4 patients, IL-6: 4 patients, IFN-γ: 5 patients) resulting in the generation of in vivo primed TIL. Twenty-three patients received $CD8^+$ TIL obtained by capture in anti-CD8-monoclonal antibody coated flasks ($CD8^+$ TIL). All patients received 96 h repetitive weekly infusions of IL-2; 48 of the patients received IFN-α administered subcutaneously on days 1 and 4 of the IL-2 infusion. A single treatment cycle consisted of three or four consecutive weeks of IL-2 therapy followed by 2 or 3 wk of rest off all therapy. Thirty-four percent of patients had an ECOG performance status of 0, and the remaining 66% were ECOG 1, and the median age was 57. The clinical responses to treatment are demonstrated in Table 3. Overall at the time of publication, there has been an objective response rate of 34.6% including 9% CR, and an overall median duration of response of 14 months, (range 0.8^+–64^+). The actuarial survival was 65% at 1 yr, and

Table 3
UCLA Trials of TIL/IL-2 Immunotherapy for Metastatic Renal Cell Carcinoma (n = 55)

	No. (%)
Response	19 (34.6)
CR	5 (9.1)
PR	14 (25.5)
Response Duration	Months
All	14 (0.8+–64+)
Median Survival	Months
All	22 (2–70+)
Responders	N/A (2–63+)

From Figlin RA, Pierce WC, Kaboo R, et al. Treatment of metastatic RCC with nephrectomy, interleukin-2 and cytokine-primed or CD8(+) selected tumor infiltrating lymphocytes from primary tumor, *J. Urol.,* **158** (1997) 740–745.

43% at 2 yr with an overall median survival of 22 months (range 2–70$^+$). This compares favorably to the predicted survival without immunotherapy of the patients based on ECOG performance status, prior nephrectomy, number of metastatic sites, recent weight loss, and prior cytotoxic therapy *(77)*.

Cellular immunotherapy with TIL, combined with low-dose IL-2 with or without IFN, has demonstrated promise in single institution trials. The overall response rate of 34.6% seen in 55 patients treated at UCLA compares favorably with the overall response rate of 15% with high-dose IL-2 alone. This has formed the basis for a phase III trial randomizing patients to low-dose IL-2 plus CD8$^+$ selected TIL *(78)* (prepared at a centralized GMP facility) vs low-dose IL-2 alone. All patients underwent radical nephrectomy to obtain tumor for TIL expansion. One hundred sixty patients were randomized (81 TIL/IL-2; 79 IL-2 alone), but 20 of these patients received no treatment postnephrectomy because of surgical complications *(4)*, operative mortality *(2)*, or ineligibility for IL-2 therapy *(14)*. The intent to treat analysis demonstrated objective response rates of 9.9% versus 11.4% and 1-yr survival rates of 55% vs 47% respectively, and was unaffected by TIL treatment. However, it should be noted that of the 72 patients eligible for TIL/IL-2, 33 (41%) received no TIL because of cell-processing failure. This inability to prepare TIL compares quite unfavorably to other single-institution experience *(74)*, and leaves the question of benefit of the addition of TIL to IL-2-based therapy still quite open. At the present time, it may not be technically feasible to consistently and effectively deliver TIL-based treatment in a multiinstitutional fashion. At UCLA, we continue to offer TIL/IL-2 to patients when appropriate.

Having patients undergo nephrectomy in order to prepare adoptive immunotherapy brings forth controversy in the management of metastatic RCC. There have been single institution reports of up to 40% of patients with metastatic RCC undergoing nephrectomy, subsequently failing to receive planned systemic immunotherapy because of perioperative morbidity/mortality, or deterioration of patient due to progressive disease *(79,*

80). In our experience, 89% of 62 patients received their planned systemic therapy (TIL/IL-2) postradical nephrectomy despite some of these patients having to undergo complicated operations including resection of caval thrombus, partial hepatectomy, and splenectomy *(74,81)*. Other single-institution experience supports these more favorable outcomes *(82,83)*. The phase III multiinstitutional TIL trial likewise showed a 12.5% postop failure to receive planed therapy *(78)*. It should be noted, however, that in these series, patients are carefully selected for this "aggressive" approach on the basis of such parameters as performance status, and lack of brain metastases. However, studies of IL-2 used alone for the treatment of metastatic RCC fail to show benefit in patient with poor performance status. At the present time, removing the primary tumor prior to planned systemic immunotherapy can be considered in select patients, especially when part of investigational trials which utilize this tumor tissue to prepare novel therapies, i.e., cell therapy, vaccines.

Therapy with TIL/IL-2 in the adjuvant setting after metastasectomy has been explored in a small feasibility trial of 22 patients including one with RCC *(84)*. TIL were successfully prepared for all participants and this ongoing trial will now focus on subjects rendered disease-free postmetastasectomy.

Attempts have been made to understand the biologic basis of response to TIL/IL-2 therapy in patients with mRCC. In vitro studies show that the immune status of the responding patients as measured by the pretreatment $CD56^+$ cell population and a serum factor able to augment in vitro PBL proliferation and cytotoxicity identified responding patients to immunotherapy *(85)*. This data strongly suggest that the immune status of the patient before immunotherapy may in part determine the outcome of therapy. Among various factors tested, responders did not differ significantly from nonresponders in number of TIL-infused, TIL phenotype, TIL cytokine mRNA expression, or in vitro cytotoxicity.

Further measures to better understand the biologic basis of response to adoptive immunotherapy need to be defined. The future role of TIL for the treatment of mRCC will depend on improvements in our understanding of the immune process in the cancer-bearing host. For example, antitumor activity may be hindered by impaired T-cell receptor signaling function, and immunosuppressive cytokines *(86,87)*. There are several possible avenues of improvement of TIL therapy most important of which may rely on reversing the immunosuppressive environment, and the identification of tumor-associated antigen targets (*see* Subheading 6.5.).

5.1. TIL-Based Gene Therapy

Gene therapy is a technique that generally involves the insertion of a functioning gene into a cell to correct an inborn genetic error, replace a defective/mutant gene, or to provide a new or improved function to the cell.

Antigen specific lymphocytes have attributes that make them attractive as a vehicle for delivery of a beneficial molecule to a targeted site. It is possible to expand them by many orders of magnitude in vitro in response to IL-2 or antigen or both. The use of IL-2 in vivo can lead to further proliferation of transfected cells and to prolonged cell survival. Most promising of all is their ability to express recombinant protein *(88)*.

Cytokine genes have been selected for TIL transduction in hopes of delivering high concentrations of cytokine to the local tumor environment, presumably increasing efficacy and decreasing systemic toxicity. There are numerous possibilities for the introduction

of cytokine genes into TIL. The first cytokine gene selected by investigators at the NCI was for TNF *(50)*. This cytokine is effective in the treatment of established murine tumors *(89)*, but highly toxic in human trials. Investigators have found that retinoic acid (RA) can lead to upregulation of gene expression in human TIL retrovirally transduced with the TNF gene *(90)*. Production of TNF was increased about twofold after treatment with RA, which has implications for TIL-based gene therapy.

Investigators have transduced T lymphocytes with the gene for IL-2 *(91)*. These cells were able to proliferate in the absence of exogenous IL-2 and still maintain effector function. This constitutive production of IL-2 by T lymphocytes may be an alternative method to prolong cell survival and possibly augment antitumor response of adoptively transferred cells.

Labeling a cell with a marker gene allows for the tracking of that cell in the body after its infusion. There have been two studies using gene-marked TIL for the treatment of mRCC *(92,93)*. Neither demonstrated selective homing of TIL at the tumor site. In the study at UCLA, both TIL and activated PBL were genetically marked using vectors that differed in their nucleotide sequences. Both marked TIL and PBL could be detected in peripheral blood up to 99 d postinfusion. Both cell populations were detected in 6:9 tumor biopsies. There was no preference seen in tumor trafficking of TIL, which could be found in biopsies of muscle, fat, and skin at greater accumulation than in tumor. These labeling studies however demonstrated that a gene could be inserted and expressed in TIL which went on to have long-term survival in the circulation.

We have recently reported the use of autologous RCC tumor line infected with the IL-2 gene via an adenoviral vector (RCC-Ad-IL-2) as a potent immune stimulant to propagate cytotoxic TIL in vivo *(94)*. Compared to standard TIL growth conditions in exogenous IL-2, TIL grown in the presence of the RCC-Ad-IL-2 had enhanced $CD4^+$ $CD8^+$ populations, enhanced TCR use, augmented HLA restricted and tumor specific cytotoxicity, and a unique cytokine profile with upregulation of IL-6 and GM-CSF. This work has implications for trials utilizing TIL propagated in vitro in the presence of RCC-Ad-IL-2.

6. OTHER THERAPEUTIC CELL POPULATIONS

6.1. Activated T Cells from Tumor-Draining Lymph Nodes

Tumor-draining lymph nodes (TDLN) presumably contain sensitized precursor, but not fully functional effector T cells that can generate Ag specific CTL. Preclinical murine models have shown therapeutic efficacy against established metastases using the adoptive transfer of TDLN that had been cultured ex vivo in low-dose IL-2, and anti CD3 *(95)*. Cross linking of the TCR with anti-CD3 triggers a signaling cascade resulting in T-cell proliferation, and cytokine synthesis *(96)*. These lymphocytes demonstrate nonspecific cytotoxicity against tumor and secrete GM-CSF and IFN-γ when restimulated in vitro with tumor cells *(97)*. Patients with mRCC have participated in early phase clinical trials using in vivo tumor vaccine primed TDLN harvested and then activated/expanded in vitro with anti CD3 monoclonal antibody and IL-2 *(98)*. Lymphocytes were infused IV with concomitant administration of IL-2. Of 12 patients with RCC, there were two complete, and two partial responses. The majority of activated lymphocytes released GM-CSF and IFN-γ in an MHC-restricted manner in response to autologous, but not allogeneic tumor. This approach has demonstrated encouraging results in small numbers of points with metastatic RCC.

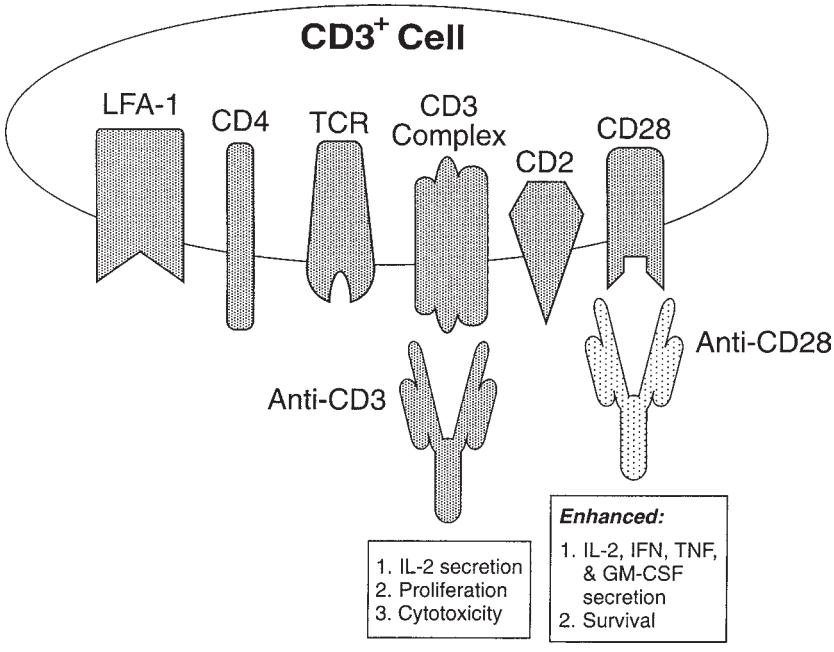

Fig. 3. Anti-CD3 anti-CD8 coactivation of T cells.

6.2. T-Cell Receptor Activated T cells (TRAC)

T-cell receptor activated T cells (TRAC), are manufactured by the ex vivo stimulation of PBL by anti-CD-3 mAb and high-dose IL-2 (100 IU/mL). They possess both NK and LAK-type cytotoxicity patterns (non-MHC restricted) and produce Th-1 type cytokines *(99)*. In murine models, TRAC were found to be more effective in the reduction of liver metastases than a similar number of adoptively transferred LAK cells *(100)*, and produced 20-fold higher cytotoxicity than LAK *(101)*. Further murine studies comparing anti-CD-3 activated $CD4^+$ vs $CD8^+$ subsets and cyclophosphamide timing, showed best results by the infusion of activated $CD4^+$ cells with IL-2 given 4 d after cyclophosphamide (at greatest WBC nadir). This sequence produced the greatest antitumor effects and survival, and Th-1 cytokine release in response to original, but not unrelated syngeneic tumor *(102)*. Clinical trials in advanced cancer patients have been reported *(103,104)*.

6.3. Anti-CD3/Anti-CD28 Coactivated T Cells (COACTS)

Because crosslinking of the TCR with anti-CD-3 may trigger a signaling cascade, other signals are likely needed for optimal immune activation and avoidance of anergy. These costimulatory signals are provided by interaction of CD28 or CTLA-4 receptor on T cells by anti-CD28 mAb or B7.1 and B7.2 (CD80 and CD 86) *(105,106)* (Fig. 3). Co-stimulation of T-cells lead to enhanced proliferation and stabilization of mRNA for a variety of Th-1 cytokines; enhanced chemokine production *(107)* and improved resistance to apoptosis due to induction of BCL-x *(108)*. Preclinical murine models of COACTS demonstrated specific cytotoxicity against a B16 tumor model *(109)*. A Phase I trial in patients with refractory cancers showed COACTS to be a feasible and safe approach, and induced immune modulation *(110)*.

6.4. Monocyte-Derived Tumor Cytotoxic Macrophages (MAC)

Peripheral blood monocytes obtained by pheresis can be cytokine activated in-vitro with IFN-γ to obtain monocyte derived tumor cytotoxic macrophages (MAC). These cells demonstrate cytotoxicity against malignant cells, and tumor bearing animal studies have demonstrated efficacy of the adoptive transfer of these cells *(111)*. There have been small numbers of patients treated with MAC in clinical trials *(112)*. The lack of efficacy in human trials of MAC will likely discourage further investigation of this cell population, and exploration of a related population of dendritic cells (DCs).

6.5. Dendritic Cells (DCs)

Dendritic cells (DCs) are the primary APC responsible for stimulating T-cell-mediated immune response *in situ (113,114)*, including antitumor immunity. DCs are bone marrow-derived leukocytes that lack cell surface markers typical for B, T, NK, or monocyte/macrophage lineage. We and others have recently described techniques for propagating large numbers of DC from PBL with the aid of the cytokines GM-CSF plus IL-4 *(113,115,116)*. These culture-derived DC arise from monocyte precursors which upon exposure to GM-CSF and IL-4 proliferate and acquire the form of mature DC. These cultured cells exhibit all of the important morphologic, phenotypic, and functional features APC that are crucial for the stimulation of both CD4 and CD8 T-cell subsets. Specifically, they express high levels of MHC class I and II, adhesion molecules (ICAM-1, LFA-3) and other important costimulatory molecules (CD40, CD80, CD86) that are essential to the process of proper antigen presentation. We have also demonstrated, via a novel phase I dose escalation trial, that DC can be generated in vivo by administering GM-CSF plus IL-4 subcutaneously to patients with advanced cancers *(117)*. These patients show minimally detectable DC at baseline, and have a marked increase in functional circulating DC after 7–14 d of cytokine administration. These observations will underlie further in vitro and clinical trials without the need for ex vivo processing of PBL to generate DC.

DCs have initiated new directions for the treatment of cancer. The premise behind this interest is that DC can be differentiated and expanded by ex vivo or in vivo cytokine exposure, loaded with tumor antigen(s), and used to induce a specific antitumor response. In a sense, antigen loaded autologous DC induces the patients own immune system to become a "bioreactor" in the education and expansion of tumor specific CTL. Although this approach cannot be classically characterized as adoptive therapy, it is worth mentioning in this chapter. DC-based phase I human trials have already shown promising results in patients with B-cell lymphoma, melanoma, and prostate cancer *(118–122)*. DCs can be pulsed with various forms of antigen including peptide-specific antigens *(121,122)*, whole proteins in the form of tumor lysates *(118,119,121,123)*, and even RNA encoding for antigens *(124)*. In preliminary studies at UCLA, we could consistently generate large numbers of functional DC from the PBL of patients with mRCC ($1.2 + 0.3 \leftrightarrow 10^6$ per 10cc of blood) using culture in IL-4 and GM-CSF *(125)*. When loaded with unfractionated RCC tumor proteins in the form of crude tumor lysate (TuLy), these DC induced a rapid proliferation of both $CD8^+$ and $CD4^+$ T lymphocytes, cytokine release, and enhancement of autologous tumor lysis, in a TIL-based culture system. Based on this in vitro data, we have commenced enrollment to a phase I clinical trial for patients with mRCC, using TuLy obtained from primary RCC to produce an autologous TuLy loaded DC vaccination *(118)* (Fig. 4). Thus far we have been able to consistently culture DC from patients

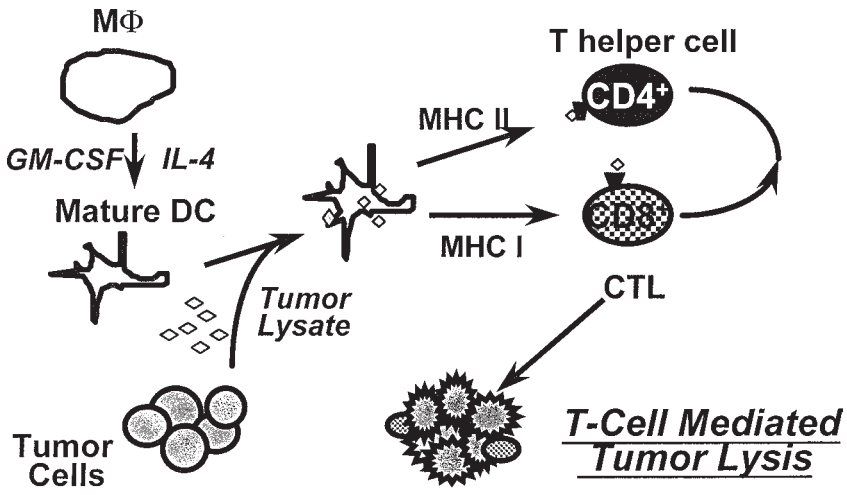

Fig. 4. Dendritic cells-based treatment of metastatic RCC.

PBMC for three consecutive TuLy-loaded DC vaccinations, and we have not observed any dose limiting toxicities.

Many ongoing DC-based studies rely on DC armed with target-specific tumor-associated antigen (TAA). The demonstration that some RCC can express common antigenic determinants that can be recognized by MHC-restricted CTL has led to efforts aimed at identifying (TAA) in human RCC. Identification of TAA is a labor intensive process which includes classification of antigens that can be used in-vitro to generate a MHC restricted CTL response *(126)*. This is followed by peptide mapping and cloning. Several candidate TAA have been identified and investigators are assessing their frequency of expression in human RCC. One such study found RAGE-1, PRAME, and gp75 mRNA expressed in adequate albeit heterogeneous frequency in surgical specimens, with sparing of nearby normal epithelium *(127)*. Another recently cloned candidate RCC-TAA is G250 *(128)*. Studies using immunohistochemical techniques and radiolabeled antibody imaging reveal that mAbG250 reacts with >75% of primary and metastatic RCC whereas no cross reactivity exists with normal kidney. Studies of the imaging and biodistribution of Iodine 131-labeled chimeric mAb G250 in patients with RCC revealed several areas of previously unrecognized metastases, and has excellent tumor localization *(129)*. These characteristics strongly suggest that mAb G250 recognizes an RCC-TAA, and is potentially an attractive therapeutic target for RCC. This suggests the possibility for TAA-based immunotherapeutic strategies for a proportion of patients with RCC.

7. CONCLUSION

Although promising in both concept and early clinical trials, adoptive cellular immunotherapy with ALT, LAK, and TIL is not yet of proven benefit in patients with mRCC. In the case of ALT, measurable tumor response has not correlated with survival advantage. The possible benefit of ALT awaits confirmation in a phase III trial using an appropriate control arm. Randomized phase III trials comparing LAK-IL-2 versus IL-2 alone have failed to show a statistically significant improvement in response rate and survival with the addition of LAK to IL-2. Adoptive therapy with TIL appears promising, although the

results of a phase III trial have failed to show difference between IL-2 alone vs TIL/IL-2. In addition, the factors determining patient response are still poorly understood. The search for effector cells with antitumor activity has continued, yielding exciting preliminary results using DC-based therapies, TDLN, and COACTS. Future clinical trials will likely also employ genetically engineered effector cells for the immunotherapy of RCC.

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24 Antiangiogenic Agents and Strategies in Renal Cell Carcinoma

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CONTENTS

1. INTRODUCTION

Although antiangiogenesis therapy recently captured the interest of the public and the research community, Folkman first described the involvement of angiogenesis in tumor growth and metastasis in the late 1960s and early 1970s *(1)*. Specifically, he and his colleagues demonstrated that tumor growth beyond a minimal volume requires the formation of new capillary blood vessels from preexisting microvessels by endothelial cell out growth (sprouting). This process involves appropriate changes in the extracellular matrix (ECM) that allows proliferating endothelial cells to migrate and form vessels within the surrounding tissue. Since that time, significant evidence has accumulated in support of the critical role that angiogenesis plays in both tumor progression and metastasis. A large body of work has identified and characterized a number of soluble factors that both stimulate and inhibit angiogenesis. The former includes vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and platelet-derived growth factor (PDGF). These factors bind to specific receptors on endothelial cells and stimulate their growth, migration, and differentiation. The observation that large primary tumors can

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inhibit the growth of their own metastases in some model systems has also led to the identification of endogenous natural inhibitors of angiogenesis. These include angiostatin and endostatin (see below).

Because of its vascular nature, the role of angiogenesis in renal cell carcinoma (RCC) growth and metastasis has been specifically examined by a number of investigators. Immunohistochemical determination of bFGF expression has, for example, been reported to be a prognostic indicator *(2)*. Increased VEGF expression has also been found in the majority of hypervascular RCCs, whereas hypovascular tumors express low levels of VEGF *(3)*. Expression of the VEGF receptor KDR has been demonstrated in the tumor vasculature of RCCs as well *(4)*. Increased levels of both serum and urine VEGF has been demonstrated in patients with localized and metastatic renal cancer and has been correlated with tumor burden *(5)*. Furthermore, decreases in serum VEGF levels have been correlated with successful therapy *(6)*. More recently, it has been demonstrated that the *VHL* gene, which is inactivated in the majority of clear cell carcinomas, inhibits the expression of VEGF, and other hypoxia inducible genes, thus providing a molecular explanation for these observations *(7)*. Finally, tumor microvessel count, which reflects the overall degree of new vessel formation, has been shown to be an independent prognostic indicator for patients with localized RCC *(8)*.

Given these observations, as well as the poor efficacy of standard therapy for RCC, a therapeutic approach that targets the tumor vasculature is particularly attractive. Currently, a number of natural and synthetic inhibitors of angiogenesis have been discovered. Although, most of the clinical trials involving antiangiogenic compounds are in their early stages, the exciting preclinical data with many has generated great patient and scientific interest. Table 1, which was obtained from the NCI's web page (http://cancertrials.nic.nih.gov/NCI_CANCER_TRIALS/zones/Pressifo/Angio/table.html), depicts the angiogenesis inhibitors in clinical trials as of February 1999. The compounds have been divided into five different classes according to their mode of action. We will discuss each class separately by detailing some properties of a representative compound. Because little data specific for kidney cancer are available, we will concentrate on the general issues and make specific reference to renal cancer only when appropriate. We will then conclude with a brief discussion of the unique challenges to clinical trial design for evaluating these compounds.

2. DRUGS THAT MODULATE MATRIX INTERACTION OR DEGRADATION

These compounds prevent newly forming blood vessels from invading the surrounding tissue. Almost all of these compounds are synthetic inhibitors of matrix metalloproteinases (MMPs). Each of the 15 known MMPs contains a chelated zinc ion at the active site and as a group they are able to cleave most proteins of the ECM and basement membrane including collagens, glycoproteins, proteoglycans, and glycosaminoglycans *(9,10)*.

By increasing blood vessel invasion into surrounding tissues, MMPs promote tumorigenicity and the metastatic potential of many cancers. For example, collagenase-1 (MMP-1) expression in colorectal cancer and stromelysin-3 (MMP-11) expression in metastatic breast cancer have been associated with poor clinical outcomes *(9,11)*. Many tumors also secrete cytokines, growth factors, hormones, and other soluble factors that can induce MMP expression in various neoplastic and nontransformed cell lines *(9,10)*. Mechanical

Table 1
Drugs that Modulate Matrix Interaction or Degradation

Drug	*Sponsor*	*Trial*	*Mechanism*
Marimastat	British Biotech; Anapolis, MD	Phase III Against pancreas, nonsmall cell lung, breast cancers	Synthetic MMP inhibitor
Bay 12-9566	Bayer; West Haven, CT	Phase III against lung and pancreatic cancers	Synthetic inhibitor of tumor growth
AG3340	Agouron; La Jolla, CA	2 trials – Phase III against nonsmall cell lung and against prostate cancers	Synthetic MMP inhibitor
CGS 27023A	Novartis; East Hanover, NJ	Phase I/II	Synthetic MMP inhibitor
COL-3	Collagenex; Newtown, PA/NCI	Phase I	Synthetic MMP inhibitor. Tetracycline derivative
Neovastat	Aeterna; Sainte-Foy, Quebec	Phase III against non-small cell lung cancer (will open later in 1999)	Naturally occurring MMP inhibitor

Direct Inhibition of Endothelial Cell Function or Response

Drug	*Sponsor*	*Trial*	*Mechanism*
TNP-470	TAP Pharmaceuticals, Deerfield, IL	Phase II against advanced cancer for adults with solid tumors; Phase I against pediatric solid tumors, lymphomas, and acute leukemias	Synthetic analogue of fumagallin protein; inhibits endothelial cell growth
Thalidomide	Celgene; Warrren, NJ	Phase III against Kaposi's sarcoma, prostate, and primary brain cancers	
Squalamine	Magainin Pharmaceuticals, Inc.; Plymouth Meeting, PA	Phase I	Extract from dogfish shark liver; inhibits sodium-hydrogen exchanger, NHE3
Combretastatin A-4 (CA4P)	Oxigene; Boston, MA	Phase I; Phase II to begin late 1999	Induction of apoptosis in proliferating endothelial cells

Inhibition of Angiogenic Factor (VEGF) Activity

Drug	*Sponsor*	*Trial*	*Mechanism*
Anti-VEGF Antibody	Genentech; South San Francisco, CA	Phase II/III against lung, breast prostate, colorectal, and renal cancers	Monoclonal antibody to vascular endothelial growth factor (VEGF)
SU5416	Sugen, Inc.; Redwood City, CA	Phase I and Phase I/II against Kaposi's sarcoma and solid tumors	Blocks VEGF receptor signaling
SU6668	Sugen, Inc: Redwood City, CA	Phase I study will open early 1999 in London	Blocks VEGF, FGF, and EGF receptor signaling
PTK787/ZK 22584	Novartis; East Hanover, NJ	Phase I against advanced cancers (Germany and UK), Phase I against gliobastoma and Kaposi's sarcoma, and Phase I/II against Von Hippel-Lindau-disease (US)	Blocks VEGF receptor signaling
Interferon-alfa	Commercially Available	Phase II/III	Inhibition of bFGF and VEGF production

(continued)

Table 1 (Continued)

Inhibition of Endothelial-Specific Integrin/Survival Signaling			
Drug	*Sponsor*	*Trial*	*Mechanism*
Vitaxin	Ixsys, Inc.: La Jolla, CA	Phase II enrollement will begin in early 1999	Antibody to integrin present on endothelial cell surface
EMD121974	Merck KcgaA; Darmstadt, Germany	Phase II/III against Kaposi's sarcoma, and brain tumors (to open later in 1999)	Small molecule blocker of integrin present on endothelial cell surface
Nonspecific Mechanism of Action			
Drug	*Sponsor*	*Trial*	*Mechanism*
CAI	NCI; Bethesda, MD	Phase II/III against ovarian, non-small cell lung, and renal cell cancers	Inhibitor of calcium influx
Interleukin-12	Genetics Institute; Cambridge, MA	Phase I/II against Kaposi's sarcoma and solid tumors	Upregulation of interferon gamma and IP-10
IM862	Cytran; Kirkland, WA	Phase III against AIDS-related Kaposi's sarcoma	Unknown mechanism

events such as cell-to-cell contact and cytoskeletal-ECM interactions also induce MMP expression *(9,10)*. In RCC, kidney fibroblasts stimulate MMP-2 and MMP-9 production by renal carcinoma cells presumably by the paracrine production of specific cytokines and growth factors *(12,13)*. The increased ratio of MMP-2 and MMP-9 expression to their natural inhibitors has also been directly correlated to the progression of RCC *(14)*. Furthermore, MMP-2 expression has been directly correlated with renal cancer metastasis and patient survival *(15,16)*.

Inhibition of MMP activity has been associated with inhibition of tumor growth and metastasis. Tissue inhibitors of metalloproteinases (TIMPs), which are natural MMP inhibitors, have been shown to repress angiogenesis and tumor metastasis *(9,10)*. A ribozyme which specifically inhibits MMP-9 has also been shown to inhibit lung metastasis in an animal model system *(17)*. Development of a clinical strategy based on inhibition of MMP activity has been aided by the identification of a number of small molecule inhibitors of these enzymes of which the most extensively studied are batimastat and mari-mastat *(10)*. Batimastat has been shown to directly inhibit endothelial cell migration and by blocking the structural organization of new blood vessels *(18)*. The low solu-bility of batimastat led to the identification of marimastat, an orally available analog, for further clinical development *(10)*.

In a phase I trials, it was demonstrated that marimastat is well absorbed and rapidly detected in the bloodstream reaching a concentration sufficient for in vitro MMP inhibition between 1–2 h after administration *(19)*. The main toxicity of marimasatat is musculoskeletal pain and polyarthritis, which is dose limiting. Although no objective responses have been reported, modest declines in tumor-marker rise have been observed and correlated with prolonged survival *(20)*. Although marimastat is currently in phase III trials, its effect in patients with RCC has not yet been investigated. As shown in Table 1, a number of additional clinical MMP inhibitors have also been identified and are in clinical trials, as well. Once again, no trials specific for renal cell cancer have been reported with these agents either.

3. DRUGS THAT DIRECTLY INHIBIT ENDOTHELIAL CELL FUNCTION OR RESPONSE

The second class of compounds inhibits the function of proliferating endothelial cells. Of these, TNP-470 has been the most extensively studied in humans *(21)*. It was the accidental contamination of an endothelial cell culture that led to the identification of fumagillin as an inhibitor of angiogenesis *(22)*. Fumagillin is a naturally secreted product of the fungus Aspergillus fumigates fresenius *(22)*. Subsequently, TNP-470 (AGM-1470), a compound fifty times more active in inhibiting endothelial cell proliferation than fumagillin, was synthesized *(22,23)*. In vitro, TNP-470 was shown to inhibit endothelial cell proliferation in a dose-dependent manner, probably by inhibiting the activation of the cyclin-dependent kinases cdc2 and cdk2, and by inhibiting pRB phosphorylation *(24, 25)*. In mouse models, TNP-470 was effective in decreasing the growth rate and angiogenesis of Renca renal carcinoma *(26)*. Similarly, TNP-470 was able to reduce pulmonary and hepatic metastatic foci of intravenously inoculated Renca *(27)*. Finally, systemic treatment of mice bearing tumors derived from human RCC resulted in significant decreases in tumor growth and metastases *(28)*.

We recently completed a phase II study of TNP-470 in patients with refractory metastatic renal cancer. Although we observed only one objective response amongst 33 evaluable patients, we did observe prolonged stable disease in six patients *(29)*. Because the trial was not designed to assess disease stabilization by the drug as an end point, it is not possible to determine whether this observation is relevant.

Like TNP-470, angiostatin is also an inhibitor of endothelial cell proliferation. Angiostatin was first isolated from the serum and urine of tumor-bearing mice and was demonstrated to be effective in inhibiting both neovascularization and growth of metastases in mice bearing Lewis-lung carcinoma *(30)*. Structurally, angiostatin is 38-kDa protein composed of the first four triple loop disulfide-linked domains of plasminogen, known as kringle domains *(31)*. Although the mechanism by which angiostatin is generated in vivo is unknown, it appears that tumor cells do not express angiostatin. It is possible, however, that tumor cells produce proteases and provide a suitable local environment in which circulating plasminogen is cleaved to generate angiostatin *(32,33)*. The mechanisms by which angiostatin inhibits angiogenesis are also unknown, although a putative receptor was recently identified *(34)*. In vitro, human angiostatin inhibits both endothelial cell proliferation and endothelial cell migration, but fails to inhibit proliferation of other cell types *(35)*. Purified angiostatin has been shown to suppress primary tumor growth in six different tumor models *(36)*. Additional studies, including clinical studies with angiostatin have been hampered by a lack of soluble, correctly folded, biologically active protein in sufficient quantities. Currently, a number of different methods of genetic engineering are being investigated to produce sufficient high-quality angiostatin for clinical trials.

Endostatin, like angiostatin, was also isolated and purified from the urine of tumor-bearing mice and is also a highly active specific inhibitor of endothelial cell proliferation and angiogenesis *(37)*. Microsequence analysis revealed that endostatin is identical to the C-terminal fragment of collagen XVIII. Currently, the proteases and other factors involved in endostatin formation are unknown. The mechanism by which endostatin mediates inhibition of angiogenesis is also not well defined. In mouse models, systemic administration of endostatin resulted in the near complete suppression of tumor-induced

angiogenesis. Furthermore, repeated therapy with recombinant endostatin induced a dormant state in several experimental tumors without the development of drug resistance *(38)*. A recent model demonstrated that the growth of the highly vascularized RCC was suppressed by systemic administration of endostatin *(39)*. Phase I clinical trials of endostatin are slated to begin in fall 1999.

4. DRUGS THAT INHIBIT ANGIOGENIC FACTOR ACTIVITY

The third class of antiangiogenic compounds is compounds that inhibit the activity of proangiogenic factors. Most of these compounds function by interfering directly with VEGF or by inhibiting VEGF receptor signaling. As noted above, VEGF is overexpressed in RCCs and thus this signaling system is an attractive therapeutic target. VEGF is a potent mitogen which induces endothelial cell migration, invasion, and in vitro formation of tube-like structures at picomolar concentrations *(40,41)*. Two endothelial cell VEGF receptors have been identified, KDR (also known as flk-1 and VEGFR2) and flt-1 (also known as VEGFR1) *(42,43)*. In addition, VEGF induces the expression of plasminogen activator, plasminogen activation inhibitor-1, and interstitial collagenases which promote the degradation of the basement membrane and ECM and enables endothelial cells to migrate into the surrounding environment *(40)*. Angiogenic activity of VEGF is synergistically increased by bFGF, which induces VEGF receptor expression in endothelial cells *(44)*.

Inhibition of VEGF activity has been accomplished by the use of a neutralizing monoclonal antibody *(41)*. VEGF exists in four isoforms of which two are soluble proteins and two are tightly bound to the ECM. Antibodies against the two soluble isoforms prevent VEGF from interacting with its receptors and thus inhibit mitogenesis and migration of endothelial cells in culture *(41)*. A second anti-VEGF antibody specific for an N-terminus epitope inactivates receptor bound VEGF at the endothelial surface and may enable specific targeting of activated endothelial calls *(45)*. The anti-VEGF neutralizing antibody inhibits tumorigenesis and metastasis in a number of human cancer models including breast, fibrosarcoma, gastric, and colon cancer models *(41,46,47)*. Clinical trials of this antibody, including trials in metastatic renal cell cancer have been initiated (Table 1).

Another strategy to inhibit VEGF signal transduction has been to develop small molecule inhibitors of the VEGF receptors flt-1 and flk-1/KDR. Flt-1 and flk-1/KDR are receptor tyrosine kinases that respond differently to VEGF binding. Flt-1 does not exhibit significant tyrosine phosphorylation in response to VEGF and is thought to play a role in the structural organization of blood vessels. In contrast, flk-1/KDR is strongly phosphorylated in response to VEGF and regulates proliferation and differentiation of endothelial cells *(48,49)*. It is thus believed that flk-1/KDR directly mediates angiogenesis and vascularization, whereas flt-1 contributes to the structural stability of the newly developed blood vessels. In support of this hypothesis, dominant negative flk-1/KDR receptors were shown to inhibit angiogenesis and growth of glioblastoma, breast, ovarian, and lung cancers in animal model systems *(50,51)*.

Recently, the SUGEN corporation has developed novel, synthetic compounds that are potent and selective inhibitors of flk-1 tyrosine kinase activity *(52)*. The lead compound for further clinical development is SU5416. This drug inhibits VEGF-dependent mitogenesis of human endothelial cells and the growth of melanoma, lung cancer, glioma, prostate carcinoma, mammary carcinoma, and fibrosarcoma subcutaneous tumors in mice *(53)*.

Initial phase I trials have suggested that SU5416 is well tolerated, but may increase the frequency of thrombotic events *(54)*. Other inhibitors developed by Sugen (Redwood City, CA) block not only VEGF receptor signaling, but also bFGF and EGF receptor signaling and thus may circumvent some of the redundant endothelial cell mitogenic signaling cascades present in tumor systems. Further studies of these compounds in patients with metastatic renal cancer are eagerly awaited.

Another compound that blocks the activity of proangiogenic factors is interferon-***a*** (IFN-***a***). IFN-***a*** inhibits endothelial cell proliferation and migration by inhibiting VEGF and bFGF induced activity *(55,56)*. Also, IFN-***a*** and -***b*** downregulate bFGF expression by 60-70% in renal carcinoma, as well as other cancers *(57)*. IFN-***a*** therapy of renal carcinoma has been a widely studied and leads to objective responses in about 10% of patients with metastatic RCC *(58)*. IFN-***a*** clearly has multiple mechanisms of action and it is not clear whether any of its clinical activity is due to its antiangiogenic activity. However, the time to response with IFN-***a*** therapy may be quite long and up to 60% of patients have been reported to experience "stable disease" *(59,60)*. These observations are consistent with an antiangiogenic effect, but clearly additional trials would be necessary to conclusively demonstrate such an effect.

5. INHIBITION OF ENDOTHELIAL-SPECIFIC INTEGRIN/SURVIVAL SIGNALING

The fourth class of antiangiogenic compounds is compounds that inhibit endothelial-specific integrin/survival signaling. Because angiogenesis depends on the adhesive interaction of endothelial cells with the ECM, targeting the endothelial cell-surface molecules involved in recognizing and interacting with this matrix provides another possible strategy for inhibiting tumor angiogenesis. Integrins are a family of transmembrane glycoproteins that mediate cell–matrix interactions. Integrins have further been shown to play a role in tumor formation and metastases *(61)*. During tumor angiogenesis, expression of the adhesion integrin ***a***v***b***3 is increased fourfold *(62,63)*. The ***a***v***b***3-integrin is a recep-tor for ostepontin, which is a matrix component important in both neovascularization and tumor angiogenesis *(64)*. Inhibition of ***a***v***b***3-integrin binding by a monoclonal antibody (Vitaxin, LM609) or other means induces apoptosis of proliferating endothelial cells *(65)*. In animal models, such approaches block tumor growth and metastases of several human tumors, including melanoma, breast, colon, pancreas, and lung carcinoma *(65–67)*. Clinical trials of the Vitaxin monoclonal antibody are slated to begin in 1999.

6. NONSPECIFIC MECHANISM OF ACTION

The final class of antiangiogenic compounds is compounds with nonspecific or unknown mechanisms of action. Of these, the most developmental work has been performed with Carboxyamidotriazol (CAI). CAI is an inhibitor of receptor gated calcium channels resulting in the inhibition of phospholipase C-***g*** and phospholipase A_2 phosphorylation *(69,70)*. Blockade of this pathway prevents the release of archadonic acid which has been correlated with inhibition of malignant proliferation and metastasis *(71)*. In vitro, this has been shown to lead to inhibition of tumor cell mobility and invasion *(72)*. CAI has also been shown to inhibit angiogenesis by inhibiting bFGF stimulation of endothelial cell proliferation, adhesion, motility, and tube formation *(71)*. In mouse models, CAI inhibited

subcutaneous growth and pulmonary metastases of a variety of human cancer cell lines *(73–75)*. In phase I studies, CAI has been well tolerated with dose-limiting toxicities of asthenia and reversible cerebellar ataxia *(69)*. In these trials, minor responses and disease stabilization was observed in a number of patients with metastatic RCC.

Another angiogenic inhibitor with multiple effects is the pleitropic cytokine interleukin 12 (IL-12). IL-12 is produced by macrophages and B-cell lymphocytes, and exhibits potent antitumor effects in mouse model systems *(76–78)*. Although the principal mechanism of action for IL-12 is thought to be stimulation of an immune response against the tumor, there is some evidence that an antiangiogenic effect is also operative. First, treatment of mice with IL-12 leads to destruction of tumor vessels by polymorphonuclear cells *(77)*. Second, IL-12 induces the expression of interferon-***g*** (IFN-***g***) by lymphocytes and much of the IL-12 effect appears to be mediated by this secondary cytokine *(77,79–81)*. IFN-***g***, like IFN-***a*** discussed above, has potent antiangiogenic activity. This activity is caused by induction of Interferon Inducible Protein-10 (IP-10), a known in vivo antiangiogenic agent *(81)*, as well as inhibition of VEGF and MMP-9 activity *(80)*.

IL-12, with or without IL-2, has been found to be extremely effective in animal models of RCC *(82)*. Clinical development of IL-12 has been hampered by unusual pharmacokinetics in which a small initial dose induces rapid tachyphalaxis to subsequent doses *(83)*. Initial trials in advanced RCC revealed very low objective response rates, but approximately 70% of the patients exhibited no progression of the disease *(84)*. As with the TNP-470 studies, the contribution of the drug to this observation can not be determined (see Clinical Trial Design below), but suggests that additional studies may need to be pursued.

7. CLINIC TRIAL DESIGN ISSUES FOR EVALUATING ANTIANGIOGENIC COMPOUNDS

Clinical evaluation of novel anticancer compounds generally follows at least a 3-step trial mechanism referred to as phase I, II, and III trials. The objective of phase I trials is to determine tolerability, dose, and schedule of the new agent. Phase II trials aim to determine whether a compound has sufficient activity in a particular malignancy to warrant further investigation. This usually means that a small number of patients are evaluated for development of a surrogate end point that theoretically correlates with patient benefit. For most cytotoxic compounds, the surrogate end point is objective response as measured by radiologically determined tumor shrinkage. It should be noted here that the typical objective response, namely 50% reduction in the sum of orthogonal two-dimensional measurements, is a rather arbitrary number that usually, but not always, correlates with symptom improvement and prolonged survival. Finally, phase III trial objectives are to determine whether a compound has a definitive impact on measures of patient benefit, which is usually determined by survival, but may also be determined by quality of life or other end points.

Evaluation of antiangiogenic compounds in renal cancer does not present any unusual obstacles during phase III trials. A promising agent could presumably be tested in a randomized fashion against some standard, such as IL2, and survival assessed. Phase I trials do raise some issues. The most important is how to arrive at the recommended dose and schedule to be evaluated in phase II trials. Traditionally, doses are escalated until the maximum tolerated dose is reached and one dose level lower is considered the recom-

mended phase II dose. This may not be necessary for antiangiogenic agents. Ideally, one would like a dose that leads to maximal biologic effect. Unfortunately, the ideal marker for maximal biologic effect is usually not available for most new agents. One potential solution is to simply choose a dose that leads to sustained serum levels of active drug that are sufficient to inhibit angiogenesis in vitro. It is likely, however, that the dose and schedule chosen during initial phase I trials will require further refinement following the initial phase II trials of a novel agent. Although there are a number of additional issues to consider in phase I trials of antiangiogenic agents, we are focusing our attention here on renal cancer, and will discuss further only the special difficulties encountered during phase II evaluation of these agents in this specific malignancy.

It is recognized that antiangiogenic therapy may lead only to tumor stabilization and growth inhibition. This may lead to significant patient benefit and prolongation of survival without inducing frank tumor shrinkage. The traditional surrogate end point of tumor shrinkage may therefore not be valid. If objective response is used as the primary end point in a standard 2-stage phase II trial, there is a high probability that one would then falsely conclude that the drug is not worthy of further investigation (statistically speaking, a large ***b*** error is introduced). The simplest approach would be to simply change the criteria of "response" from objective tumor shrinkage to objective tumor shrinkage *or* stable disease for a defined period. The difficulty with this approach is the highly variable nature of metastatic kidney cancer. Surgical and cancer registry series clearly document a 5–10% five-yr survival for patients with metastatic disease even in the absence of effective therapy *(85)*. Furthermore, patients with such indolent disease tend to be overrepresented at referral institutions that typically perform these trials. At our institution, for example, we have noted that about 30% of patients will maintain stable disease for 16 wk after beginning any phase II agent, including cytotoxic agents that do not induce any objective responses. One could simply "set the bar higher" in terms of proportion of patients that need to experience stable disease in order to deem the agent effective. Unfortunately, there is no *a priori* value that can logically be chosen for this set point. Even the use of prognostic scores to predict the expected rate of stable disease will not be helpful since the accuracy of these scores is rather poor *(86,87)*. These considerations, as well as the uncertainties inherent in distinguishing stable disease from slowly progressive disease, leads to a high probability that an ineffective drug will be recommended for further development (statistically speaking a large ***a*** error is introduced).

One theoretical advantage to targeting the tumor vasculature for therapy is its genetic stability. Preclinical data suggest that resistance is therefore not easily induced *(38)*. This allows a novel approach to determine whether the observed stable disease in an individual patient is likely caused by the drug or by naturally indolent disease. The scheme depicted in Fig. 1 classifies patients as "responders" if there is a typical objective response (i.e., tumor shrinkage) or if stable disease is documented during therapy and progressive disease is documented during a drug holiday. Thus the surrogate endpoint is either tumor shrinkage or stable disease as defined in Fig. 1. Others have suggested a similar approach, but require evidence of progressive disease prior to beginning therapy with the antiangiogenic agent or require a period of observation prior to beginning therapy. The former approach is plagued by inconsistent and nonstandardized data prior to entry into a formal protocol and the latter is plagued by poor patient acceptance. The scheme in Fig. 1 is likely to be better accepted by patients because all patients are immediately treated with the new

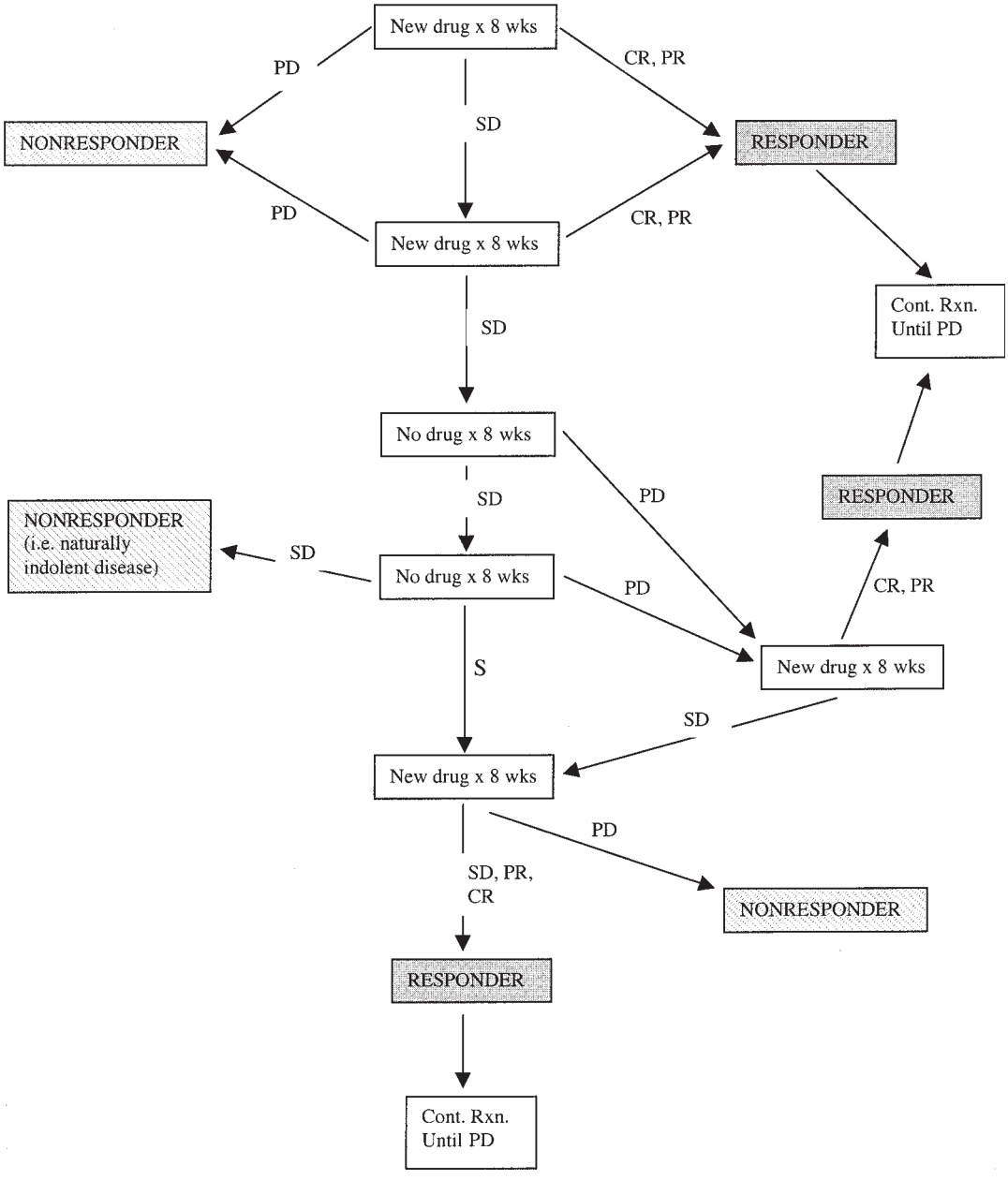

Fig. 1. Possible scheme for lacrifying as "responders" patients who experience prolonged stable decrease while being treated with an investigational antiaagence agent.

agent. In addition, a period of drug therapy is usually accompanied by a least some toxicities. Thus, in our experience, continued treatment in the face of only stable disease engenders at least some conflicting feelings amongst both patients and physicians. In clinical trial parlance, there is true equipoise in terms of the relative advantages of continuing or discontinuing therapy.

The major difficulty with the outlined scheme is that the time frame between enrollment of a patient and subsequent classification as a responder may be quite long. Thus, if standard two stage accrual methods are followed, accrual to the second stage may be unduly delayed until all the patients in the first stage are evaluated *(88)*. An alternative approach is to use a randomized discontinuation scheme *(89)*. With this approach, all patients are treated with the novel antiangiogenic agents. At a specified evaluation time-point (we suggest 16 wk) patients are randomized to continue or discontinue the drug and continued stable disease is assessed at a second evaluation time-point (once again we suggest another 16 wk). Ideally this would be performed in a placebo double-blind manner. Once again, this allows all patients to receive the new agent and performs the randomization at a time when true equipoise is likely to be present in both the patient, as well as the physician. Although space prevents a full discussion, such a trial design requires far fewer patients to be randomized than a standard randomized trial design *(89)*. For example, an increase in the stable disease rate from 20% to 50% at the second evaluation point could be detected with only 30 patients per group with a one-sided α-error of 0.05 and a power (1-β) of 0.8.

Finally, the principle biologic effect of these antiangiogenic therapies is to prevent new vessel formation. Because this can be assessed by microvessel staining, clinical trials can be designed to use decreased tumor microvessel density as the primary surrogate end point. Clearly this would require a one or more biopsies that may not be clinically indi-cated, but in our experience the majority of patients are motivated enough to agree to core-needle biopsies. Typically one would determine vessel density in a biopsy specimen after a specified treatment period and compare this to vessel density before treatment in each individual. However, because the *intra*-individual (or *intra*-tumor) variability of vessel staining is as high or higher than the *inter*-individual variability, there is no par-ticular statistical advantage to a paired test. Instead, one could randomize patients to receive one biopsy either prior to therapy or after a specified treatment period and then simply compare the mean values in these two groups. Once again, study sizes can be quite modest. For example, estimating that the standard deviation of the measurement is 0.75 of the mean, using an α error equal to 0.1, and a power (1-β) of 0.8, a 50% decrease in microvessel density in the late biopsy group can be detected with 17 patients per group.

Although other trial designs are possible, it is our contention that such novel phase II designs with novel surrogate endpoints will be necessary in order to quickly and efficiently evaluate which one of the large number of putative antiangiogenesis agents is suitable for further development and evaluation in phase III trials.

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25 Therapy for Patients with Uncommon Histologic Varieties of Renal Cell Carcinoma

Robert J. Amato

CONTENTS

1. INTRODUCTION

Renal cell carcinoma (RCC) is a common urologic malignancy affecting approximately 40,000 people annually in the United States *(1)*. One-third to one-half of the patient population with RCC has metastatic disease at presentation. Although the overall survival rate at 5 yr is approximately 50%, patients with metastatic disease have a median survival rate between 7 and 11 mo. Tumor grade and cell type are strong predicting factors of survival in patients with and without metastatic disease *(2)*.

The majority of renal cancers arises from the proximal tubule epithelium, has a characteristic clear or granular cell appearance by light microscopy, and is referred as RCC. These tumors constitute approximately 80% of the renal cell neoplasms, with a male: female ratio of 2:1 and a peak number of cases occurring in the sixth decade. Cytogenetic studies have shown that the most common abnormality, which occurs in about 96% of the cases (including hereditary ones), is the loss of one allele in the smallest overlapping region of chromosome 3p13 *(3–5)*.

Classical RCC is insensitive to cytotoxic agents, as well as radiation therapy. The role of progestational agents in the treatment of metastatic RCC is limited with most investigators accepting a minimal activity in the 5% range, although higher response rates can be achieved in selected groups of patients *(6,7)*. Adjunctive nephrectomy does not prolong survival or induce significant regression in patients with metastatic RCC, but it may enhance the efficacy of systemic therapy *(8–10)*. The most promising agents used in the

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treatment of RCCs are biologic response modifiers, with single agent interferon-alfa (IFN-α) showing response rates in the 15% range (CR+PR) and combination therapies with IFN-α/interleukin-2 (IL-2)/5-Fluorouracil or IFN-α retinoids showing response in the 30–40% range, most of them usually seen in patients with soft tissue and/or pulmonary metastases *(11)*.

2. COLLECTING DUCT CARCINOMA

Collecting duct carcinoma is a subtype of RCC, which has received attention in the recent literature, accounting for 1–3% of renal neoplasms *(12–28)*. The first report was published in 1976 *(12)*. Fleming and Lewis *(16)* defined diagnostic criteria and established collecting duct carcinoma as a separate histological entity arising from the renal medulla and later invading the cortex. The presence of a medial tumor that has a mixed solid and tubulopapillary histological pattern with an infiltrating tubular component and a marked desmoplastic reaction is required for the diagnosis of this entity. The cells are cuboidal with relatively bland nuclei oriented toward the lumen with clear, faintly granular, or eosinophilic cytoplasm lining a fibrovascular stroma *(3)*. Supportive evidence is the finding of dysplastic changes in the collecting duct epithelium adjacent to the tumor *(16)*. The cell of origin is the collecting duct epithelium. Collecting duct carcinoma has been designated in the World Health Organization classification of kidney tumors as Bellini's duct carcinoma *(29)*. Ultrastructural examination may reveal cells with scant microvilli, tight junction complexes, and a moderate number of mitochondria *(16)*. Immunohistochemistry reveals positively for markers of collecting duct epithelium, such as epithelial membrane antigen, high (and low) molecular weight keratin and peanut lectin *(16,18)*. These findings are more closely related to urothelial carcinoma rather than to cortical papillary RCC *(17)*, justifying, in the mind of several investigators, trials with chemotherapeutic agents used in transitional cell carcinoma.

The clinical behavior of collecting duct carcinoma is not well defined, because few cases are reported to date. However, collecting duct carcinoma appears to often affect a younger population and carry a worse prognosis than classical RCC, following an aggressive clinical course *(16,18,19,21,23,24,26)*. Collecting duct carcinoma frequently presents as a renal mass, most commonly causing macroscopic hematuria and flank pain.

Radiographically, it usually presents as a centrally arising mass with "preservation" of the contour of the kidney *(18,21)*. Recently, cytogenic studies revealed a pattern of the abnormalities: monosomies 1,6,14,15,22 *(20)*, loss of heterozygosity (LOH) of 8p (50% of the cases studied) *(27,28)*, a finding that is also seen in 23% of cases of transitional cell carcinoma of the urinary bladder *(28)* and LOH of 13q (50% of the cases) *(27)*, all of which are distinct from the cortical papillary kidney tumors, exhibiting trisomy 17 and tri- or tetra-somy 7 *(29–31)* and from the nonpapillary RCCs characteristically presenting deletion in the short arm of chromosome 3 *(32–37)*.

Most of the clinical information in collecting duct carcinoma comes from single-case reports. Dimopoulos et al. *(21)* reported in 1993 in a retrospective review at the University of Texas MD Anderson Cancer Center experience with collecting duct carcinoma. Twelve patients were included in that report (male:female ratio of 2:1, median age at diagnosis of 43 yr). Eight of twelve patients presented with metastatic disease; the four patients with nonmetastatic disease at diagnosis underwent radical nephrectomy with hilar lymphadenectomy, but in all cases the tumor recurred in less than 1 yr. Seven patients

were treated with various chemotherapy regimens, more commonly with MVAC (Methotrexate, vinblastine, doxorubicin, and cisplatin) combination chemotherapy, a urothelial type of treatment. Only one patient showed a minor response of short duration. One patient treated with 5-Fluorouracil, IFN-α, and mitomycin-C had an objective stabilization for 16 mo. Six patients were treated with a combination of IL-2 and IFN-α 2b, one of whom achieved a good partial remission and was then rendered surgically free of disease for 30 mo.

A combination of IFN-α, 5-Fluorouracil, and cisplatin (FAP) is an ongoing phase II study in patients with metastatic unresectable collecting duct carcinoma of the kidney. IFN-α 2b (Intron) has only minimal activity in metastatic urothelial neoplasms, but the combination of Intron with 5-Fluorouracil (a combination with synergistic activity first shown in colon carcinoma) resulted in 30% response rate in our initial report for patients being treated for chemotherapy refractory urothelial tumors. The combination of Intron, 5-Fluorouracil, and cisplatin was then tested at University of Texas MD Anderson Cancer Center in patients failing or not able to receive MVAC chemotherapy *(38)*. In these 28 patients, a 57% response rate (95% CI; 48-71%) (CR: 7%, PR: 50%, PD: 43%) was seen, with moderate morbidity and most of the patients being able to receive their treatment as outpatients. The major dose-limiting side effect was thrombocytopenia and stomatitis in these heavily pretreated patients. This regimen is interesting because first it is an active regimen in metastatic urothelial tumors, but it also combines agents IFN-α and 5-Fluorouracil with documented activity even in RCC. Therefore, the possibility of a mixed tumor or of a tumor difficult to be differentiated between RCC-classical or papillary, and collecting duct carcinoma will not compromise the initial treatment of the patient.

3. SARCOMATOID RENAL CELL CARCINOMA (RCC)

Sarcomatoid RCC is an uncommon histologic variant of RCC. Sarcomatoid RCC constitutes approx 1.0% to 4.8 % of the total number of renal parenchyma tumors *(39–42)*. First described by Farrow et al. in 1968 *(39)*, this tumor consists of a small or large degree of clear cells or granular cells with pleomorphic spindle cells and/or malignant giant cells resembling sarcoma.

This sarcomatoid component stains positively for cytokeratin (AE1/AE3) in 85–94% of the cases, demonstrating the epithelial nature of the spindle cells. Desmosomal junctions noted by ulstrastructual studies as sarcomatoid RCC confirm the epithelial nature of the neoplasm *(43–45)*. Sarcomatoid RCC is a distinct entity characterized as a highly proliferative carcinoma as shown by a high proliferative cell nuclear antigen and intense silver staining for nucleolar organizer regions *(46)*. It is also associated with worse prognosis than nonsarcomatoid RCC, higher metastatic and local recurrence rate, and shorter survival time *(39–42)*. Sarcomatoid RCC cannot be distinguished radiologically from clear cell RCC *(47)*.

Recently, it was suggested that failure to express assembled major histocompatibility class I complexes on the tumor cell surface may be responsible for their insensitivity to immune recognition and to IFN-α treatment. This defective major histocompatibility class I expression has been demonstrated both in tissue and in a cell culture derived from sarcomatoid RCC *(48)*.

This tumor has received only scant attention; only four relatively large series (all retrospective) have appeared in the literature describing the clinicopathologic characteristics

of these patients *(39–42)* and little is known about the disease's sensitivity to cytotoxic agents, radiation or biologic therapy.

Tomera et al. *(40)*, in their retrospective review, studied 13 patients with pathologic characteristics of sarcomatoid RCC (1% of their RCC population). Twelve had nephrectomy and one had a biopsy only. The majority had extensive disease at the time of surgery (time of diagnosis). Only two patients had disease confined within the renal capsule, five patients had perinephric fat invasion (stage II), one patient had regional lymph-node involvement (stage III), and five patients had distant metastatic disease. Survival was discouraging. Only one patient with stage I disease was alive, without evidence of disease 49 mo after resection. The remaining patients died of metastatic disease, with a median postoperative survival of 6.3 mo (range 1–14 mo).

Sella et al. *(41)* reviewed 44 cases of sarcomatoid RCC (patients with any percentage of sarcomatoid element in their pathology) (4.8% of 920 patients with RCC seen at University of Texas MD Anderson Cancer Center over a 10-yr period). Twenty-five of the 44 patients had distant metastases at diagnosis (56.8%) (stage IV) and 19 patients had stage I–III disease. The survival of sarcomatoid RCC patients was discouraging; median survival of 6.6 mo (range: 2–81 mo) compared to a median survival of 19 mo (range 1–308 mo) for the nonsarcomatoid RCC patients. This difference is evident also in a stage-by-stage comparison. Stage IV sarcomatoid RCC patients had a median survival of 4.5 mo (range: 2–30 mo) with no long-term survivors, whereas the median survival of the stage IV nonsarcomatoid RCC patients was 9.4 mo (range: 1–127 mo; $p < 0.05$) and 40 of them (9.9%) were alive at the time of analysis. For stages I–III disease, patients with sarcomatoid RCC had a median survival of 28.5 mo vs 46.2 mo for the nonsarcomatoid RCC ($p < 0.05$).

The retrospective review of 44 patients with sarcomatoid RCC at the University of Texas MD Anderson Cancer Center showed that patients treated had a survival advantage over the ones who did not receive systematic therapy (median survival 13.0 mo vs 3.8 mo). No uniform therapy was used, but there was some suggestion that patients treated with doxorubicin-containing regimen had some therapeutic effect. The only responses in the 31 treated patients, were two complete responses in patients treated with CYVADIC (cyclophosphamide, vincristine, adriamycin, dicarbazine, cisplatin), who were also the only long-term survivors. Four patients were treated with IFN-α and did not respond.

Pathologic evaluation of the same patients (42 out of the 44) showed that pathologic stage and percent of necrosis at the sarcomatoid area were predictors of survival *(49)*. The percent of sarcomatoid component at the primary site (nephrectomy) was predictor only for stage I and II patients. Although none of the four patients treated with IFN-α responded to treatment, their median survival was 41 mo, probably reflecting less-aggressive tumors.

A more recent retrospective review of the experience at the institut Gustave-Roussy *(42)* included 14 patients with sarcomatoid RCC and greater than or equal to 60% of sarcomatoid elements in their pathological evaluation. Thirteen of the 14 patients had initial nephrectomy. Ten patients were treated with chemotherapy, eight of whom with doxorubicin-containing regimens. Four patients were treated with IFN-α, none responded. The only responses were four partial responses in, all after doxorubicin-containing regimens (CYVADIC: 1, DECAV [dicarbazine, etoposide, cyclophosphamide, adriamycin, vincristine]: 2, DI [doxorubicin, ifosfamide]: 1) suggesting some activity of doxorubicin in this disease.

A combination of IFN-α, doxorubicin, and ifosfamide in a phase I/II study at the MD Anderson Cancer Center in patients with metastatic RCC with any sarcomatoid component with control of the primary tumor, preferably by nephrectomy is ongoing. IFN-α is an active regimen in RCC; objective responses have been reported with as little as 1 mIU/d subcutaneously *(50)*. It does appear that chronic dosing is superior to intermittent higher doses with respect to tolerability and antitumor activity. Previous experience at University of Texas MD Anderson Cancer Center with combination chemotherapy (5-fluorouracil, adriamycin, cisplatin, mitomycin-c [FAMP]) in patients with metastatic RCC showed a response rate of 27%. A phase II study compared FAMP chemotherapy to FAMP chemotherapy alternating with IFN-α and showed similar response rates in both arms of the trial (12%) *(51)*. Our interpretation of the low response rates seen in the phase II trial was that we failed to reproduce the original trial design; that is continuous exposure to IFN-α while we gave adequate chemotherapy. A subsequent trial of circadian infusion of floxuridine (FUDR) for 14 d along with continuous exposure to low-dose IFN-α (2 mIU/m2/d subcutaneously) shows responses in 30% of the patients with metastatic RCC *(52)*.

In vitro studies suggest synergistic effect of IFN-α, a plus doxorubicin against human tumors *(53–55)* including RCC. Cole et al. showed that IFN-α enhances the major histocompatibility class I antigen expression and results in growth inhibition in a small-cell lung cancer cell line and its doxorubicin-selected multidrug resistant variant *(54)*. IFN-α was also able to reverse adriamycin resistance in a human colon carcinoma cell line *(55)*. The optimal antiproliferative effects of combined IFN-α and doxorubicin are realized when maximal concentrations of IFN-α and prolonged cell exposure time for both IFN-α and doxorubicin were employed *(53)*. Different phase I/II studies have been conducted combining IFN-α with doxorubicin in human malignancies *(56–58)*. Myelotoxicity was a problem, but was better tolerated when doxorubicin was given as a weekly dose *(50)*. Ifosfamide has activity in RCC *(59)* and shows synergism with IFN-α in human nonsmall cell lung cancer xenografts *(60)*.

4. ONCOCYTOMA

Oncocytomas have a characteristic pattern on gross examination. Tumors are usually highly regular, well-circumscribed lesions with an encapsulated appearance; on bisection, the characteristic uniform ruddy tan to mahogany brown color is classically found. Except in the largest lesion, little or no evidence of necrosis or hemorrhage is seen *(61, 62)*. Occasionally a central stellate scar is observed. This gross pathologic character is in marked contrast to RCCs, which commonly show frequent areas of necrosis or hemorrhage and typically are orange-yellow. The characteristic microscopic feature of renal oncocytoma is a highly regular, homogenous cytoplasm with marked eosinophilia.

One of the more controversial aspects of the clinical phenomenon of renal oncocytoma is its putative malignant potential. Although it was originally considered to be invariably benign, there has been substantial controversy regarding its potential for malignancy, particularly in cases in which more pleomorphic histopathologic patterns have been seen in the tumor. One of the controversies relates to the appropriateness of grading oncocytomas. A series from the Mayo Clinic *(61)* used a three-grade system in a survey of 90 tumors diagnosed as oncocytoma. Of the 28 patients with grade 2 tumors, two specimens contained tumor within regional lymph nodes, and four patients died of metastatic disease.

The clinical cohort from which such outcome analyses are derived is established by the histopathologic definitions. Criticism of the Mayo Clinic study, suggests that sampling of the tumors was limited in several instances, which may have precluded recognition of more aggressive histiotypes in other areas of the tumor *(61)*. Investigators have pointed out that tumors with oncocytic features are containing a typical histopathologic features, which the Mayo Clinic study considered a reason for justifying the need to assign a grade 2 or grade 3 designation, to such tumors, should not be interpreted as oncocytomas *(62,63)*. There are marked deficiencies in the medical literature regarding correlation of histopathology with clinical outcomes.

Numerous retrospective reviews of renal oncocytoma have been published in the literature, summarized in a recent review *(64)*. It is relevant to note that numerous instances of bilateral lesions have been reported *(64)*. Because oncocytoma has only an extremely remote likelihood of metastasizing, it must be inferred from this information that these are multifocal lesions. Oncocytomas have been associated with RCC elsewhere, approximately 10% of the time *(65)*. Consequently, it is imperative to recognize that sampling of one lesion does not define the pathology for the other lesions. The biologic potential of oncocytomas is extremely favorable for the patient. The mode or extent of surgical intervention is controversial, whether a complete nephrectomy or nephron sparing surgery should be entertained.

5. ANGIOMYOLIPOMA

In 1951, Morgan and Associates *(66)* described a renal tumor that contained vasculature of a thick-walled nature with variable amounts of intermixed tissue of smooth muscle, adipose tissue, or both.

Angiomyolipoma occurs in the absence of tuberous sclerosis, most frequently in women between age of 35–60 yr. Cases often present with flank pain, hematuria, and occasionally, massive hemorrhage necessitating emergent surgery *(67)*. Because such masses are characteristically hypervascular lesions, that share many characteristics with RCC, identification of zones of extremely low density (reflecting adipose tissue) within the masses allows the diagnosis of angiomyolipoma to be made pathognomonically in the vast majority of cases *(68)*.

The clinical behavior of angiomyolipoma has been the focus of considerable attention. No clearly defined evidence of distant metastatic disease has ever been reported. Reports of dissemination to adjacent lymph nodes has been described.

Patients who present with flank pain and hematuria usually have large lesions. Nephrectomy is curative and is important in the presence of massive hemorrhage. Selective angioinfarction or nephron-sparing surgery has been used *(69)*. Asymptomatic single lesion can be observed by a CAT scan evaluation every year to determine the trend of enlargement. Symptomatic hematuria requires surgery, when anatomically possible, partial nephrectomy. Massive life-threatening hemorrhage requires selective angioinfarction with follow-up surgery either renal sparing or a total nephrectomy.

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26 Palliation in Patients with Advanced Renal Cell Carcinoma

The Interface with Antineoplastic Therapy

Donna S. Zhukovsky

CONTENTS

1. RELEVENCE OF SYMPTOMS

1.1. Magnitude of Problem

In 1999, 30,000 people in the United States will be diagnosed with cancers of the kidney and renal pelvis *(1)*. Although most cases are sporadic, populations at increased risk for developing renal cell carcinoma (RCC) include those with familial syndromes such as hereditary clear cell renal carcinoma, hereditary papillary renal carcinoma, and von Hippel-Lindau (VHL) syndrome, as well as patients with autosomal dominant polycystic kidney disease, tuberous sclerosis, and end-stage renal disease who develop acquired cystic disease of the kidney *(2)*.

The majority of individuals with RCC come to medical attention because of symptoms. Retrospective data suggest that more than 70% of patients are symptomatic at diagnosis, with most studies citing figures of 70–80% *(3–13)*. Kidney-specific symptoms of hematuria, flank pain, and/or flank mass are the most common presenting complaints *(5,8–11,14,15)*. Nonrenal symptoms as presenting manifestations caused by paraneoplastic phenomena and metastases are also common and nonspecific, leading to delays in diagnosis *(5,16)*. Up to 37% of patients have metastatic disease at presentation *(4,7,8–13, 15,17,18)*. For patients with localized disease, paraneoplastic symptoms typically remit with successful treatment of the tumor, i.e., nephrectomy and return with disease recurrence or progression *(14,15,19–23)*. Adding to the broad spectrum of symptoms experienced

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by this patient population are the treatment-related toxicities of surgery, radiation, chemotherapy, and biologic response modifiers *(2,3,24–30)*.

1.2. Prognostic Implications

The prognostic factors predicting outcomes for patients are discussed in Chapter 9. With improved radiologic imaging techniques, the incidental detection of this tumor has increased over the last several years *(6,9,11,18)*. Most *(6,11,17,18)*, but not all *(9)*, investigators report that incidentally detected tumors are diagnosed at an earlier stage than are symptomatic tumors, with attendant survival benefits. Skinner *(10)* reports survival differences between asymptomatic patients, symptomatic patients without metastases, and patients symptomatic from metastases, but no significant prognostic relations of individual presenting symptoms or laboratory abnormalities. In contrast, Gelb *(31)*, on behalf of the Union International Contre le Cancer and the American Joint Committee on Cancer, states that symptomatic presentation, significant weight loss, poor performance status, and other laboratory findings are associated with a less favorable clinical course. Weight loss, hypercalcemia, elevated alkaline phosphatase, and possibly, elevated serum ferritin were negative predictors in the setting of metastatic disease.

1.3. Treatment Implications and Quality of Life

The high incidence of symptoms at presentation with RCC raises questions about their impact on antineoplastic therapy, quality of life, and caregiver burden. In the general cancer population, symptom number typically increases as patients traverse the disease course, with the most common symptoms also being the most severe *(32,33)*. Both symptom number and symptom distress increase psychological morbidity, impair quality of life *(34)*, and have the potential to impact on compliance with antineoplastic and other treatments. Optimization of antineoplastic therapy and symptom control decreases patient and caregiver burden and improves quality of life.

2. CLINICAL MANIFESTATIONS OF RCC

2.1. Renal Manifestations

At diagnosis, approximately two-thirds of patients have at least one urologic symptom *(5,8–11,14,15)*. Gross hematuria is the most common, affecting 50–60% in most retrospective series *(4–14)*. It is followed by flank pain in 20–50% and a painless flank mass in 20–30%. The classic triad of these three symptoms, the *sine qua non* of RCC, is much less common at 5–10%. Details are displayed in Table 1 *(4–14)*.

2.2. Extrarenal Manifestations

RCC is one of the great masqueraders in medicine. This reflects its numerous and nonspecific extrarenal manifestations, which include a variety of paraneoplastic phenomena, as well as symptoms from metastatic sites of involvement *(4,5,7,8,10,13–15,19–22, 33,36,38–40)* (*see* Table 2). Lung and bone are the most common metastatic sites *(8,43)*. Extrarenal manifestations can be categorized as nonspecific systemic symptoms commonly seen with other malignancies such as weight loss, fever, elevated sedimentation rate, hepatic dysfunction and anemia, symptoms caused by eutopic hormone production (i.e., a hormone or polypeptide similar in structure to naturally occurring hormones produced by the kidney), and symptoms caused by ectopic hormone production (i.e., hormones

Table 1
Incidence of Renal-Specific Symptoms at Diagnosis

Ref #	*# of Patients*	*Age (yr)*	*Male (%)*	*Study Type*	*Flank Pain (%)*	*Gross Hematuria (%)*	*Flank Mass (%)*	*Classic Triad (%)*	*At Least One Urologic Symptom (%)*	*No Symptoms (%)*	*Comments*
14	273		65	retrospective review	42	18	62				Histologically confirmed diagnosis
4	96	55.7 (23–77)	70	retrospective review	32	35	35	6		28	Histologically confirmed diagnosis
5	18	28.3	72	retrospective review	50	56	11		72	6	Histologically confirmed diagnosis, 78% with adenocarcinoma, restricted to patients ages 20–40 at diagnosis
6	74 asymptomatic (236 total)	59.8	67	retrospective review						31	Age and gender distribution refer to asymptomatic patients only
15	30	61.2 (55–74)	67	retrospective review	33	23	13	0	57		Diagnosis histologically confirmed in 25 of 30 patients
7	27			retrospective review	26	22 (includes microscopic hematuria)				30	
8	577			retrospective review					68[a]	1	
9	235			retrospective review	30	49	15	1	72	28	Histologically confirmed diagnosis
10	309		67	retrospective review		60		9		7	
11	164			retrospective review	49	62	40	8		15	Histologically confirmed diagnosis
12	135	58.2	60	patient interview on hospitalization	20	39	1			18	Histologically confirmed diagnosis, symptom causing patient to seek medical attention
13	400	58.7 (15–83)	67	retrospective review	24	32	36	5		21	Histologically confirmed diagnosis

Table 2
Incidence of Extrarenal Symptoms At Diagnosis

Ref #	*Fever (%)*	*Weight Loss (%)*	*Fatigue (%)*	*Hypertension (%)*	*Varicocoeles (%)*	*Erythrocytosis (%)*	*Anemia (%)*	*Hypercalcemia (%)*	*Hypocalcemia (%)*	*Abnormal Liver Functions (%)*	*Elevated ESR (%)*	*SXS Related to Metastases*	*Comments*
14	16	31				5	28					8	amyloidosis 3%
4	14	30	32	21	4	13	23	12 (33 patients tested)	6 (33 patients tested)	7	50 (58 patients tested)	25	
5		6		6			6						
15	37	36	37	10		3	27	7		10	40		
7	4	4			7	7		4		4			
8		12	6	1	1								
10					2	3	21	4				10	
13	13	22	14			5	7	4				15	cough 6%, neurologic sxs[a] 3%, vascular sxs 2%, gynecologic sxs 2%

[a]sxs= symptoms.

Table 3
Hormonal and Paraneoplastic Manifestations of Renal Cell Carcinoma

• Hypertension	• Acquired dysfibrinogeninemia
• Erythrocytosis	• Cushing's syndrome
• Anemia	• Amyloidosis
• Hypercalcemia	• Neuromyopathy
• Fever	• Elevated HCG
• Constitutional symptoms	• Elevated prolactin
• Hyperglycemia	• Elevated ferritin
• Nonmetastatic hepatopathy (Stauffer's syndrome)	• Elevated prostaglandins

not normally produced by the kidney) *(15)*. Symptoms may be caused by more than one mechanism. For example, hypercalcemia may occur because of the presence of bone metastases or secondarily, to a parathormone-like substance *(15,20,37)*. Many symptoms and syndromes remit with removal of the tumor and, as such, may be considered paraneoplastic phenomena. It is important that the relationship of symptoms to paraneoplastic phenomena be recognized, as their presence does not imply the invariable presence of metastases and are not contraindications to potentially curative nephrectomy *(19)*. The paraneoplastic syndromes are listed in Table 3, and are discussed in Chapter 8.

2.3. Treatment-Related Manifestations

Surgery, radiation, and chemotherapy and procedural interventions *(9)* are associated with acute and chronic pain syndromes *(44–46)*. Acute postoperative pain, as well as postnephrectomy syndrome, are common sequelae of nephrectomy. With the latter, pain occurs because of interruption of the L1 nerve, with numbness, fullness, and heaviness in the flank, anterior abdomen, and groin. When associated with dyesthesiae, pain may be neuropathic in nature. Diagnosis of this syndrome requires exclusion of paravertebral tumor recurrence. Potentially beneficial interventions include use of an abdominal corset, transcutaneous electrical nerve stimulation, and adjuvant medications such as amitriptyline for the control of neuropathic pain *(46)*. Postinfarction syndrome, another treatment-related morbidity, is described in almost all individuals undergoing angioinfarction nephrectomy. This syndrome includes abdominal pain, nausea, vomiting, diarrhea, and fever *(24)*.

The side effects of radiotherapy are site-specific and well detailed in textbooks of oncology, as are the numerous and diverse side effects of cytotoxic chemotherapy, hormonal therapy, and biologic therapies. The biologic therapies, in particular, have become an important treatment approach for unresectable and metastatic RCC. Associated side effects are somewhat distinct from those of cytotoxic chemotherapy in that constitutional symptoms and flu-like symptoms of nausea, vomiting, and diarrhea predominate. For the interleukin-based therapies, mental status changes, thrombocytopenia, and cardiopulmonary toxicities are prominent. Hypotension, capillary leak syndrome, and features of septic shock may occur. Many of the toxicities are of moderate to severe intensity and dose-related *(2,3,24,25,27)*.

Table 4
Importance of a Pain-Specific Diagnosis and Control

- Disease detection and direction of antineoplastic therapy
- Choice of appropriate analgesic therapies
- Detection of impending neurologic catastrophes
- Optimization of quality of life

3. SYMPTOM EVALUATION

3.1. Importance of a Pain-Specific Diagnosis and Control

Pain in the cancer patient may occur because of tumor, antineoplastic treatment, or from unrelated reasons. Tumor pain is the most common, affecting 62–78% of patients, often from bone involvement, compression of a viscus or neural impingement. Next most common is pain caused by chemotherapy, radiation, or surgery at 19–25%. Least common, but of great psychological impact when mistaken for cancer-associated pain, is pain such as arthritis that is unrelated to the cancer or its treatment. Unrelated pain occurs in 3–10% *(47,48)*.

A pain-specific diagnosis yields many advantages over blind symptom control, as listed in Table 4. In a study of pain consultations done in hospitalized cancer patients, the pain-specific diagnosis led to an undiagnosed etiology of pain in 64%. Metastatic tumor was the most common cause. New neurologic diagnoses were established in 36% and unsuspected infection in 4%. In 18%, the pain-specific diagnosis resulted in a change in antineoplastic therapy as new chemotherapy, radiation, or surgery *(55)*. In addition, pain control facilitates patient compliance with disease evaluation and antineoplastic therapy.

3.2. Pathophysiologic Mechanisms of Pain in Renal Cancer

Pain related to RCC is classified as nociceptive or neuropathic, with mixed syndromes being common *(48,50–54)*. Both start with activation of peripheral nociceptors by mechanical, thermal, or chemical stimuli, followed by transmission of nociceptive impulses rostrally via the spinal cord. Nociceptive pain requires ongoing stimulation for its maintenance and resolves with stimulus ablation. Somatic nociceptive pain occurs with activation of nociceptors in skin, bone, or deep tissue. It is typically well localized, constant, or intermittent, and described as aching, gnawing, throbbing, or crampy. Visceral pain, the other subtype of nociceptive pain, occurs with stretch or distension of intrathoracic or intraabdominal viscera. It is less well localized than somatic pain and qualitatively described as deep squeezing, pressure, or colicky pain. Nausea, vomiting, and diaphoresis are frequent concomitants. Visceral pain may be referred to distant cutaneous sites that are tender to palpation. Colicky abdominal pain from bowel obstruction is a classic example of visceral pain. Pharmacologically, most nociceptive pain responds well to treatment with traditional analgesics such as acetaminophen, nonsteroidal antiinflammatories, and opioids *(48,51,52,54)*.

Unlike nociceptive pain, neuropathic pain may persist after ablation of the underlying stimulus. It occurs from damage to peripheral and/or central nervous system tissue. The most common cause of neuropathic pain in the cancer population is from nerve infiltration or compression by tumor. Other causes include chemotherapy-related neuropathies, radiation-induced myelopathy and plexopathies, and viral injury such as seen with post-

Table 5
Anatomic Locations of Kidney Pain

Site of collection system distension	*Location of pain*
renal pelvis	ipsilateral costovertebral junction ± ovary or testicle
ureteropelvic junction	anterior superior iliac spine
midureter	middle of Poupart's ligament
utereterovesical ureter	suprapubic region ± scrotal or labial skin, medial thigh

herpetic neuralgia. Neuropathic pain is an unfamiliar experience to most individuals. Common qualitative descriptors include burning, electric shock-like pain, painful numbness and formication, the sensation of insects crawling on one's skin. Often more difficult to treat than nociceptive pain, neuropathic pain may benefit from use of adjuvant analgesics such as tricyclic antidepressants or anticonvulsants *(48,51–53)*.

3.3. Kidney Pain

The vast majority of kidney pain is visceral, although localized or referred pain may occur with extension of the disease process to the parietal peritoneum. Mechanisms of kidney pain include distension of the renal capsule or the urinary collecting system, extravasation of urine into adjacent tissues, inflammation, ischemia, traction, and displacement on the renal pedicle or involvement of adjacent organs. For obstructive syndromes, intensity of pain is related to rate of distension, as well as associated extravasation of urine *(58)*.

3.4. Anatomic Location of Kidney Pain

The kidney is innervated by branches of T12-L2, but also has a rich autonomic supply from the celiac and aorticorenal ganglia, the upper lumber sympathetic trunk, and vagal parasympathetics. Kidney pain, therefore, is experienced in the ipsilateral costovertebral angle, but may be referred to the inguinal and thigh regions, as somatic afferents from these areas enter the spinal cord at the same level as the renal autonomic afferents. Ureteral innervation is derived from the renal, spermatic, or ovarian and hypogastric plexuses, accounting for the differential referral pattern depending on the segment of ureter affected. Anatomical sites of referral are described in Table 5. Afferent sensory fibers follow the course of vagal afferents, resulting in the nausea, vomiting, and diminished intestinal peristalsis that accompanies some pain syndromes *(58,59)*.

3.5. Assessment of Pain

The purpose of a pain assessment is to delineate the underlying etiology and contributing factors necessary to synthesize a working diagnosis and treatment plan directed at all factors contributing to the suffering of the individual in pain *(50,51,53)*. The history,

Table 6
Pain History-Specifics

- Location
- Temporal features
  - onset
  - duration
  - pattern
- Relieving/exacerbating features
- Qualitative descriptors
- Analgesic history (previous drug trials)
  - duration of use
  - dose schedule
  - onset of drug action
  - duration of drug action
  - associated side effects
- Objective pain measurement
  - categorical scales
  - numeric rating scales
  - visual analogue scales
  - other

consisting of general medical and pain-specific components, is the mainstay and directs the subsequent physical examination and diagnostic testing. During testing, pain should be aggressively treated with analgesic premedication in order to prevent exacerbation. Analgesic premedication enhances patient comfort, patient cooperation, and acquisition of quality data. Specifics of the pain history are noted in Table 6. For an accurate portrayal of the patient's pain experience, assessment of intensity includes knowledge of present pain intensity and usual, least, and maximum pain intensity over a specified period of time. Pain pathophysiology and sites of nociceptive lesions can frequently be determined from the history and confirmed on physical examination and diagnostic testing. From a more general perspective, knowledge of the patient's functional and psychosocial function provides additional information regarding impact of pain on the individual. This facilitates the development of a treatment plan targeted at all factors contributory to the patient's suffering *(50,51,53)*.

3.6. Definition of Nonpain Symptoms

Symptoms are patient reports that suggest the presence of an underlying disease or disorder. Because of their inherently subjective nature, symptoms are challenging to assess. However, use of standardized terminology and objective measurement tools facilitates symptom assessment and evaluation of clinical interventions *(60)*.

3.7. Symptom Assessment: Concordance of Patient and Proxy Reports

Symptom assessment frequently involves information obtained from family members, friends, and health-care professionals. Proxy assessment is the sole source for individuals who are unable to provide self reports because of temporary conditions, such as delirium or more durable conditions, such as dementia or language barriers. Discrepancies in patient and proxy report are well established in the literature. The best documentation is for pain, where health-care professionals consistently underestimate pain severity

relative to patient report. Discordance increases with increasing pain severity *(61,62)*. Less information is available about concordance of patient-proxy report for nonpain symptoms. Available data suggest that family caregiver report is highly correlated for pain and performances status. Unlike health-care professionals' tendency to underestimate pain intensity, family caregivers typically overestimate patient pain and disability relative to the patient's report *(63)*. In a recent multicenter study of patient and professional report, concordance was higher for physical than for psychological and cognitive symptoms. Concordance was greater for the absence than the presence of a given symptom *(64)*.

3.8. Approach to Symptom Assessment

Like pain, nonpain symptom assessment is based on history, physical, and indicated diagnostic tests to formulate a working diagnosis and treatment plan. In addition to the symptom-specific evaluation, history includes knowledge of the patient's general medical and oncologic history. Specific parameters to evaluate include frequency, intensity, associated distress and response to previous therapies (*see* Subheading 3.5.). Each symptom deserves individual evaluation, as well as evaluation of its impact on the psychosocial, functional, and spiritual domains of quality of life. Combined with physical examination and relevant diagnostic tests, symptom assessment establishes symptom pathphysiology and contributing etiologies necessary for the development of an optimal treatment plan.

3.9. Use of Symptom Measurement Tools

A variety of symptom scales or batteries have been developed to quantify symptom parameters for clinical or research purposes. Scales may be symptom-specific and unidimensional, such as numeric rating scales, categorical scales, and visual analog scales of pain intensity or symptom-specific and multidimensional, such as the Memorial Pain Assessment Card *(65)*, The Brief Pain Inventory *(66)*, and the Piper Fatigue Self-Report Scale *(67)*. Symptom batteries are designed to measure multiple symptoms in single or multiple dimensions, such as the Rotterdam Symptom Checklist *(68)* and the Memorial Symptom Assessment Scale *(65)*, respectively. Objective forms of symptom measurement also provide a means to establish accountability for symptom control *(69,70)*.

3.10. Role of On-Going Assessment in Symptom Control

On-going symptom assessment is integral to the provision of optimal care. It allows for monitoring of treatment efficacy and related side effects, detection of new or changing pathophysiologies, and opportunities for patient/family education essential to optimal patient care.

4. APPROACH TO MANAGEMENT OF SPECIFIC SYMPTOMS

4.1. Pain

4.1.1. Available Modalities

Good pain-control regimens are typically multimodal (*see* Table 7). In the renal carcinoma patient, antineoplastic therapy, and pharmacotherapy are the mainstays. They are often used in conjunction with physiatric and cognitive-behavioral therapies. Physiatric means of pain control are complementary to pharmacotherapy by using a variety of prostheses and orthoses for control of specific pain syndromes and physical therapy for the optimization of independent function and prevention of secondary, painful, myofascial

Table 7
Modalities of Pain Control

- Antineoplastic
- Pharmacologic
- Anesthetic
- Surgical
- Neuroaugmentative
- Physiatric
- Cognitive-behavioral

complications. Cognitive-behavioral therapies incorporate a variety of cognitive and behavioral approaches to modify the experience of pain and suffering. Physiatric and cognitive-behavioral means of pain control additionally benefit the treatment plan by facilitating patient empowerment and the lack of overlapping side effects with drug therapy. Of great benefit, anesthetic techniques, such as nerve blocks for regional analgesia or spinal opioids for multifocal pain and neurosurgical techniques, such as cordotomy, are indicated in a minority of patients *(70,71)*.

4.1.2. Antineoplastic Therapies

Antineoplastic interventions specific to symptom palliation in RCC include nephrectomy, embolectomy, and inferior vena cava thrombectomy, in addition to chemotherapy, biologic response modifiers, and radiotherapy *(2,24,30,71,72)*. Nephrectomy plays a role in control of renal-specific symptoms and paraneoplastic syndromes from nonmetastatic tumors. For patients with metastatic disease, it is used for the control of severe pain or hematuria when other methods are unsuccessful. Efficacy of symptom control with these approaches is not well documented. A variety of renal embolization techniques and angioinfarction nephrectomy are alternatives to surgical nephrectomy, but may be associated with relatively high rates of recurrence when used for control of hematuria *(30)*.

Radiation has established precedent for control of bone pain and symptoms related to central nervous system, pulmonary, and other metastases. Stereotactic radiosurgery for solitary brain lesions and endobronchial techniques for bronchial obstruction are beneficial in selected patients. Radiopharmaceuticals, such as radioactive strontium (^{89}Sr) are effective in relieving pain, improving quality of life, delaying the development of new metastases, and reducing the need for future radiotherapy in patients with multiple sites of bone pain. The majority of data is derived from patients with metastatic prostate carcinoma. Overall response rate (pain relief) for prostate patients is approximately 70%, with relief occurring 7 to 14 d after treatment and lasting 3 to 6 mo. Response rates for patients with metastatic bone lesions from different primary tumors is reportedly lower. Other radiopharmaceuticals under evaluation are samarium (^{153}Sm-EDTMP) and rhenium (^{186}Re-DP). Side-effect profile includes flare of bone pain lasting 36–48 h, thrombocytopenia, and leukopenia. Impending spinal cord compression or pathological fractures, DIC, and low white or platelet counts are contraindications to radiopharmaceutical use. Factors affecting cost–benefit ratio relative to other therapies include response rate, degree, and duration of pain relief and survival *(16,73–75)*.

4.1.3. Bisphosphonates

Like radiopharmaceuticals, bisphosphonates palliate multiple painful bone metastases in a variety of tumors. They also diminish the occurrence of skeletal-related events, such

as need for subsequent radiotherapy for bone pain, incidence of pathologic fractures, hypercalcemia, and development of new osteolytic lesions. Some trials have included patients with RCC. Mechanism of action is mediated at least in part by inhibition of osteoclast-mediated bone resorption. Analgesia appears to be dose dependent and may be delayed, sometimes requiring repeated intermittent dosing. At present, pamidronate, the most potent of the commercially available bisphosphonates, administered intravenously every 3 to 4 wk, is the most common regimen in the United States. Side effects tend to be mild and include transient low-grade fever, nausea, myalgia, bone pain, and mild infusion-site reactions. Symptomatic hypocalcemia is rare. Adverse gastrointestinal effects are more prominent with oral bisphosphonate formulations. Rehydration of dehydrated patients prior to administration of bisphosphonates is recommended to prevent acute renal failure. Bisphosphonate administration is contraindicated in patients with renal failure, although the risk of renal impairment in patients with normal renal function is low. In patients with mild renal impairment, some recommend that the dose and/or infusion rate be lowered *(16,75–80)*.

4.1.4. Pharmacotherapy

Pharmacotherapy is the mainstay of pain control in patients with RCC, as antineoplastic therapies are often ineffective or delayed in onset. Available drug classes include the nonopioids, the opioids, and the adjuvant analgesics. The nonopioid analgesics consist of acetaminophen, aspirin, and a wide variety of nonsteroidal antiinflammatories. Common to this group of drugs is a ceiling effect to analgesia. Continued dose escalation beyond a specified maximum for each drug does not yield additional analgesia, but does incur a risk of increased toxicity. Drugs in this group are used as single agents for mild to moderate pain and as adjuncts for moderate to severe pain. Nonsteroidal antiinflammatories are particularly beneficial for bone pain *(81–85)*.

Opioid analgesics have no ceiling. Continued dose escalation results in additional drug effect. Regular opioid use is associated with the biologic phenomena of physical dependence and tolerance, as distinct from psychological dependence (addiction). Tolerance and physical dependence are frequently confused for psychological dependence by patients, families, and health-care professionals. Tolerance is characterized by the development of resistance to drug effect with continued use. Shortening the dose interval or increasing the dose restores analgesia. In cancer patients, tolerance can be difficult to differentiate from progressive disease, which more often is responsible for dose escalation. To clinical advantage, tolerance to most drug side effects develop more rapidly than to analgesia. Physical dependence is defined by the development of an abstinence syndrome on abrupt drug discontinuation or with the administration of an opioid antagonist. Withdrawal can be prevented for most patients by administering at least a quarter to a third of the previous day's dose or by administering a dilute solution of antagonist in situations of life threatening respiratory depression. Most opioids can be administered by multiple different routes *(81–86)*.

The adjuvant analgesics are a diverse group of drugs including antidepressants, anticonvulsants, neuroleptics, corticosteroids, hydroxyzine, oral local anesthetics, and membrane stabilizers. Each has a primary nonpain indication, but also has intrinsic analgesic activity. The antidepressants, anticonvulsants, membrane stabilizers, and oral local anesthetics are used to control neuropathic pain. Corticosteroids are potent antiinflammatoires for treating bone pain, pain from epidural spinal cord compression, and pain from increased

Table 8
Principles of Pharmacotherapy

• Believe the patient
• Start 1 drug at a time; allow adequate trial
• Choose appropriate route of administration
• Provide regularly scheduled drug administration for continuous pain syndromes
• Provide supplemental doses (breakthrough or rescue doses) for breakthrough pain
• Anticipate, prevent and aggressively manage side effects
• Assess pain control in an ongoing manner
• Be easily available to patient

intracranial pressure. They have the added advantage of simultaneously treating nausea, vomiting, and anorexia in this typically multisymptomatic population. Their benefits must be balanced against the potential for the development of a significant side-effect profile. Like the nonopioid analgesics, they are frequently used in conjunction with members of other drug classes for optimal effect *(81–87)*.

Principles of pharmacotherapy are presented in Table 8. Appropriate drug selection for the pain syndrome, knowledge of opioid equianalgesic doses and pharmacokinetic properties, indications for different routes of drug administration, and side-effect management are integral to successful pharmacotherapy. On-going assessment based on good communication skills is mandatory to monitor treatment effects and to identify new pathology *(81–84)*.

4.2. Hematuria

The causes of hematuria in RCC are multiple. Except when nonspecific symptom control is the clinical goal, knowledge of symptom etiology is requisite to establishing a treatment plan. Causes directly related to the tumor include invasion of the renal collecting system by tumor, obstruction, and coagulopathies. Patients may also develop hematuria caused by urinary tract infection, calculus formation, and unrelated conditions of the bladder and prostate. Management of kidney-related hematuria by nephrectomy, embolization, and angioinfarction has been discussed under antineoplastic interventions of pain management (*see* Subheading 4.1.2.) *(2,24,30,71–73)*. Rarely, radiation has been used for the control of intractable hematuria from RCC *(73)*. Other causes are treated depending on their etiology, i.e., antibiotics for infection-related hematuria. Concurrent with disease-specific interventions, or as primary treatment, continuous bladder irrigation for dissolution, and prevention of painful clot formation, urinary tract analgesics, such as phenzopyridine and antimuscarinc agents, such as belladonna and opioid suppositories for control of painful ureteral and bladder spasms can be used. Irrigation of the urinary tract with capsaicin is under investigation. Other than clinical experience, little data exist to support or refute the use of these therapies *(88–92)*.

4.3. Delirium

Increasingly recognized as a consequence of cancer and its treatment, delirium has been reported to occur in up to 85% of terminally ill hospitalized cancer patients. Its occurrence signifies increased morbidity and mortality that frequently results in hospitalization or prolongs established hospitalization *(93,94)*. A source of distress to patients, families, and health-care professionals, delirium carries implications for patient consent to

treatment and ability to comply with treatment plan, as well as for caregiver burden. Delirium is frequently undiagnosed by the medical team or misdiagnosed as depression *(95–97)*.

Common causes of delirium in the renal cell patient and the cancer population at large include metabolic encephalopathies caused by organ failure, electrolyte disturbances, side effects of antineoplastic interventions, such as chemotherapy or neuraxial radiation therapy, drug side effects, infection, anemia and coagulopathies, nutritional deficiencies, and paraneoplastic syndromes. Opioids, anticholinergic agents, and corticosteroids are well established drug offenders. Although frequently multifactorial, a specific etiology may not be determined *(95,96,99,100)*. Of particular relevance to the RCC patient is the association of delirium with the use of biologic-response modifiers, an important treatment modality in this population *(2,3,24,25,101)*.

Treatment of delirium requires a high index of suspicion for its diagnosis. History is key, with dementia, depression, anxiety reactions, and withdrawal syndromes from alcohol or benzodiazepines among the differential diagnoses. Delirium may be superimposed on a preexisting dementia *(102)*. Evaluation is directed at detecting reversible causes. Validated measurement tools may be used to diagnose and follow the course of delirium *(104)*. Management is based on treating reversible causes when clinically appropriate and symptomatic treatment of the delirium. The latter involves general supportive management and environmental manipulation to enhance orientation (i.e., quiet, well-lit room with large, digital clock and calendar, familiar objects from home), and minimize sleep and sensory deprivation. A frequent precipitant of delirium, benzodiazepines should be avoided in the symptomatic treatment of delirium, with few exceptions. Benzodiazepines are effective in treating delirium tremens from alcohol withdrawal and as adjuncts to neuroleptics in the treatment of agitated patients. For most patients, haloperidol is the drug of choice. Its use must be monitored for the development of extrapyramidal side effects, movement disorders, and malignant neuroleptic syndrome. During the treatment process, communication with the family and patient as able to explain and educate offers additional support *(102,104,105)*.

4.4. Fatigue

Fatigue is one of the most prevalent and distressing symptoms associated with cancer and its treatment *(32–34,106)*. Also called weakness, aesthenia, and tiredness *(107)*, Portenoy proposes specific diagnostic criteria to facilitate research of this pervasive, multidimensional symptom *(106)*. The definition encompasses physical and mental correlates of fatigue and provides a basis for investigation of contributing pathphysiologic mechanisms. As for pain and other symptoms, objective measures can be used to assess fatigue and to evaluate the impact of treatment interventions *(60)*.

Major etiologic categories include direct tumor effects (i.e., brain metastases), tumor-induced products (i.e., cytokines), and tumor-accompanying factors (i.e., paraneoplastic phenomena, treatment-related side effects) *(108)*. Utilizing a different model, factors associated with the disease or its treatment, intercurrent systemic disorders, sleep disorders, immobility, and lack of exercise, chronic pain, use of centrally acting drugs, anxiety disorders, and depression have been invoked as potential causes *(106,107)*. Given the high prevalence of anemia, paraneoplastic phenomena, and use of biologic response modifiers in the renal cell population, fatigue may be a major symptom at every stage of disease. Because of the paucity of data, interventions are empirically directed at potentially reversible mechanisms as determined by individual patient assessment, in conjunction

with symptomatic therapy. Pharmacologic approaches include use of psychostimulants, corticosteroids, antidepressants, and amantadine. Nonpharmacologic approaches involve patient education, exercise, modification of activity and rest patterns, cognitive therapies, and attention to nutritional factors *(106,107)*. Establishing realistic expectations about causality of fatigue, therapeutic options and their anticipated impact is an important component of the treatment regimen *(106)*. Fatigue in the caregiver should also be addressed *(107)*.

5. INTEGRATION OF PALLIATIVE CARE INTO COMPREHENSIVE CANCER CARE

In the United States, the vast majority of cancer resources are directed at curative effects throughout the patient's disease course, with palliative care being a minor focus at the very end of life. The World Health Organization suggests a model in which palliative care starts from the time of diagnosis, in conjunction with antineoplastic efforts. In this model, the emphasis on disease-curing and palliative therapies shifts as the patient traverses the disease course *(109)*. MacDonald further supports this concept in his statement that prevention of suffering is the fourth component of cancer prevention. He suggests that palliative medicine programs be an integral component of cancer centers *(110)*. The high prevalence of symptoms in RCC at presentation and of treatment-related morbidity emphasizes the need for the dynamic interface of these integral components of state-of-the-art cancer care.

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Renal Cell Carcinoma

Molecular Biology, Immunology, and Clinical Management

Edited by

Ronald M. Bukowski, MD and Andrew C. Novick, MD

Cleveland Clinic Foundation, Cleveland, OH

Over the past ten years, much significant new information concerning the epidemiology, biology, and treatment of renal cell carcinoma has appeared. In *Renal Cell Carcinoma,* leading clinicians and researchers critically survey this enormous body of clinical, biological, and pathological knowledge, and show how it is best applied to the management of both localized and advanced renal cell carcinoma. Their discussions include full treatment of the roles of partial nephrectomy, radical nephrectomy, and laparoscopy, as well as the latest developments in molecular genetics and immune dysfunction associated with the disease. Also discussed are screening for renal cell carcinoma, its diagnosis and staging, paraneoplastic syndromes, and prognostic factors in metastatic disease.

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