Ovarian Cancer

Cancer Treatment and Research

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Cancer Treatment and Research

Foreword

Where do you begin to look for a recent, authoritative article on the diagnosis or management of a particular malignancy? The few general oncology textbooks are generally out of date. Single papers in specialized journals are informative but seldom comprehensive; these are more often preliminary reports on a very limited number of patients. Certain general journals frequently publish good in-depth reviews of cancer topics, and published symposium lectures are often the best overviews available. Unfortunately, these reviews and supplements appear sporadically, and the reader can never be sure when a topic of special interest will be covered.

Cancer Treatment and Research is a series of authoritative volumes which aim to meet this need. It is an attempt to establish a critical mass of oncology literature covering virtually all oncology topics, revised frequently to keep the coverage up to date, easily available on a single library shelf or by a single personal subscription.

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Where can you go to find quickly a recent authoritative article on any major oncology problem? We hope that Cancer Treatment and Research provides an answer.

WILLIAM L. MCGUIRE
Series Editor

Preface

Ovarian cancer is the leading cause of death from gynecologic tumors, accounting for approximately 12,000 deaths per year in the United States. It causes more deaths than from uterine and cervix cancers combined. Recent textbooks of medical oncology and gynecology discuss 5-year survival rates for patients with advanced disease (i.e. Stages III and IV) of 0 to 5%. In that up to 70% of the patients present at diagnosis with advanced disease the outlook for the ovarian cancer patient has been extremely poor. In the past five years considerable progress has been accomplished in the diagnosis and management of ovarian cancer which has had a dramatic impact on the improvement of the disease-free and overall survival of these patients.

This progress has led to a multi-disciplinary approach to the management of this disease and a major change in its natural history. As a result of more thorough staging techniques, aggressive, cytoreductive surgery and combination chemotherapy including cisplatin 5-year survival rates in patients with Stages III and IV disease have increased from an expected 0–5% to as high as 30–40%. The chapters in this book have been designed to describe and document these important advances and serve as a blueprint for continued research efforts.

The chapters concerning ovarian tumor cytogenics, antigens and stem cells include exciting, new research data which are consolidated for the first time in a single text. This information is invaluable with respect to an understanding of the molecular and cell biology aspects of the disease. The chapters concerning surgical staging, cytoreductive surgery, radiation therapy, single and multiple agent chemotherapy and intraperitoneal drug administration are definitive in their documentation of progress with these different therapeutic approaches. They will serve as an important text for future reference and presently provide the most optimal therapeutic approaches in the management of both early and advanced ovarian cancers.

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We would like to dedicate this book to our wives, Heather Alberts and Lee Surwit, whose loving support and tenacious spirits have greatly enriched our lives and have made our careers successful.

1. Prevalence and clinical significance of cytogenetic abnormalities in human ovarian cancer

JEFFREY M. TRENT

1. Introduction

Despite recent advances in therapeutic strategies, ovarian cancer remains the leading cause of gynecologic cancer death in the United States. Likewise, although a significant body of basic research data is available on ovarian carcinoma, we still lack fundamental knowledge of most of the important biologic mechanisms responsible for imparting a generally poor prognosis in this common disease. However, recently an increase in our knowledge of the basic genetic mechanisms apparently associated with the malignant state has occurred. These studies of human 'oncogenic' sequences have brought together basic scientists using molecular and cellular biologic techniques with clinical scientists skilled at interpreting the clinical features associated with the malignant phenotype. This area of cancer biology appears most likely to provide important information on the genesis and ultimately clinical progression of human cancer. The major objective of this chapter is to highlight the recent studies of chromosomal and DNA sequence alterations in ovarian cancer, in the hope that understanding these changes may provide new insights into the mechanisms responsible for initiation and progression of this disease.

2. General background: cytogenetics

Although numerous chromosomal analyses of ovarian cancer (OV-CA) have been performed for over three decades, results have differed widely (for review see [1]). The reason for the relatively frequent study of ovarian cancer by cytogenetic techniques can be explained largely from the availability of metastatic populations obtained from malignant ascites or pleural fluids. Accordingly, virtually all published reports of OV-CA have studied metastatic populations.

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Within the past few years a renewed interest in the analysis of chromosomes from ovarian tumors has occurred. These studies arise from new advances in cell culture techniques and the application of detailed chromosome-banding methods. Although this resurgence in work on OV-CA has lead to an overall increase in our knowledge of genetic changes in this disease, like earlier studies, current work reveals considerable variability. One possible reason for this variability is that the comparison of even recent studies of chromosome change in OV-CA is complicated by investigators employing several different culture methods and chromosome harvesting techniques in an effort to obtain sufficient mitotic cells for detailed analysis. Accordingly, results have ranged in the extremes, from the report of normal diploid cells [2], to the report of exclusively aneuploid cells [3]; from the report of no recognizable tumor associated chromosome change [4], to the report of a high concordance between a specific chromosomal defect and a specific histologic subtype of ovarian cancer [5].

Despite these difficulties in comparing and reviewing the cytogenetic data from OV-CA, I believe conclusive evidence exists that certain chromosomal changes are indeed freqeuntly associated with this disease. Further, it is possible that certain of these consistent chromosomal alterations may result from an underlying molecular defect.

3. Relationship of chromosome numbers with DNA ploidy measurements in OV-CA

Numerous studies of DNA ploidy in OV-CA have been performed and have revealed two major populations: 1) A near-diploid group and 2) a group with high model DNA values approaching the triploid-tetraploid range [6]. On the basis of these DNA studies, it has been suggested that those patients with near-diploid modal DNA values demonstrate a better survival rate than those with high modal *DNA* values [6, 7]. Of interest, comparisons of survival rates between patients whose tumors were studied for chromosome number have generally not supported a distinct survival advantage for patients with a near-diploid chromosome number [1]. What exactly is responsible for these seemingly contradictory results in OV-CA is unclear, although numerous possibilities exist. First, it is possible that ovarian tumors with a favorable prognosis are less suitable for cytogenetic analysis. Therefore, successful chromosomal studies in OV-CA may generally 'select' patients with a poor prognosis. A second possibility is that even short term *in vitro* culturing of tumors to obtain adequate numbers of mitoses again 'selects' a non-diploid population. However, a third, and I believe most likely explanation for the difference between DNA ploidy and cytogenetics studies resides in the different end-point measured by these two

techniques. Therefore, DNA ploidy techniques have the advantage of assaying all cells within a given tumor, without respect for morphologic or pathologic status, or equally important without respect to the growth potential of the cells analyzed. Thus, all cells (including normal reactive cells) within a given tumor will be measured by DNA content techniques. Similarly, most cytogenetic studies are also biased by utilization of a non-cellular specific selecting agent (e.g., colchicine or another mitotic arresting agent) which selects equally well normal or tumor cells. The difference seen between these methods may then relate to the size of the 'window' examined, with DNA content examining a full window of all cells within a tumor and then arriving at a 'modal value', while cytogenetic analysis selects a much smaller window composed only of those cells (normal and abnormal) capable of proliferation.

In summary, patients with OV-CA can usually be divided into a near-diploid vs. a hyperdiploid population, although there are reported differences between studies examining DNA content and chromosomal studies. Thus, while these different methods may have an important role in our understanding of the genetic changes in this disease, they may not provide information which can be readily compared.

The choice of culture method prior to chromosome analysis for study of OV-CA is of utmost importance in determining the fraction of the tumor cells available for study. A brief description of the culture methods most often utilized in cytogenetic analysis of OV-CA is presented below.

4. Culture methods

4.1. General comments

The major obstacle to successful cytogenetic analysis of human solid tumors remains the acquisition of a sufficient number of metaphases available for detailed cytogenetic analysis. Chromosomal analysis of human solid tumors in general, and ovarian cancer in specific, has involved the use of several different culture and chromosomal harvesting techniques. The majority of studies have involved the use of a 'direct' chromosome harvesting technique, a method which adds colchicine to cell populations obtained following disaggregation of a tumor, followed by immediate chromosome harvesting [8, 9]. The use of a direct method on malignant ovarian ascities provides a reasonably good means of obtaining mitoses with an overall success rate of $>50\%$ not uncommon [8]. In contrast, studies of primary tumors with the identical harvesting method virtually always is less successful, with cytogenetic success rates usually $\ll 20\%$ [10]. The principle difficul-

ties using a direct method for cytogenetic studies are two-fold: 1) metaphases obtained (particularly from solid tumor specimens) are often obtained in only a fraction of the cases studied and ordinarily are suboptimal for chromosome banding analysis, and 2) a significant admixture of normal mitoses along with tumor mitoses are often obtained. As discussed previously, this is precisely what would be expected with the use of a non-cellular specific selective agent (e.g., colchicine) which stops all cells in cycle at mitosis (both normal and abnormal).

In an effort to select a relevant tumor population prior to colchicine exposure and to overcome some of the aforementioned difficulties in cytogenetic analysis of solid tumors, several recently developed culture techniques have been introduced. A recent report by Yunis [11] reviewed several of the most commonly utilized cell culture and chromosome harvesting techniques for solid tumors, including 1) amethopterin synchronization to elongate and increase the number of tumor mitoses [12], 2) enzyme disassocation of primary tumors followed by short term liquid culture [13], 3) use of feeder layers or tissue explants to encourage *in vitro* growth of tumor cells [14], and 4) soft agar culture of tumor specimens followed by cytogenetic analysis [15]. The last method, soft agar 'cloning' is, I believe, ideally suited for cytogenetic study of OV-CA. A description of the methods of procedure for agar culture for growth of ovarian tumor cells in agar culture is provided below.

4.2. Clonogenic cell growth

The procedure followed for colony formation in agar culture is that of Hamberger *et al.* [16]. Briefly, $2\text{–}5 \times 10^5$ tumor cells are suspended in 1 ml of 0.3% molten agar containing enriched medium CMRL-1066 and 15% heat in activated (HI) horse serum. The cells are then placed into 35 mm Falcon Petri dishes over a 1 ml 0.5% agar underlayer containing an admixture of HI fetal bovine serum and growth factors. Cultures are then incubated at 37 °C with 7.5% CO_2 in a humidified air environment. Plates are examined serially with inverted microscopy at 100× magnification for evidence of cluster-colony formation. Colonies are defined as aggregates of >40 cells, clusters aggregates of 6–40 cells.

4.3. Cytogenetic procedures for tumor colony forming cells

The procedure utilized is that described by Trent and Salmon [15]. Agar cultures incubated at 37 °C are initially overlayed with 2.5 ml of culture medium containing 0.1 uM colchicine. While a 1 hour colchicine incubation

is standard, up to 16 hours exposure facilitates study of cultures with limited cellular proliferation. Following colchicine incubation, the entire plating layer (or single individual colonies using micro-pipet procedures), are exposured first to hypotonic (0.075 M KCl) and then exposed to fixtive (3:1-methanol-glacial acetic). Standard air dried slides are then prepared and cells subjected to chromosome banding [17]. Importantly, chromosome banding of colony mitoses from agar culture can be performed without interference from any residual agar remaining on the slides.

We are currently in the process of comparing a sufficient number of tumors studied by several of the previously mentioned techniques in an effort to try and establish whether substantial selection of tumor populations occurs utilizing either the agar or various liquid culture techniques. One major difference between the agar system and other liquid culture techniques is the virutal absence in the agar culture system of normal diploid mitoses, an exceedingly common finding with liquid culture methods (up to 100% of mitoses observed in many cases [18]).

Although the liquid culture techniques represented a marked improvement over the aforementioned direct techniques, these methods are clearly not selective only for tumor cell growth. In contrast, a variety of biologic techniques suggest that growth of cells in agar culture is highly selective of growth of tumor cell populations. These techniques include cytogenetic analysis [3], isoenzyme analysis [19], flow micro-fluorometric analysis [20], and production of tumors in immunosuppressed animals following injection of tumor colony forming cells (TCFUs) [20]. Despite the substantial increase in the technical sophistication required to successfully utilize the agar culture technique, evidence strongly suggests that this method provides a potentially important selective means for analyzing a high enriched tumor cell population. The major difficulty with wide spread application of this method rests in the inadequate growth of many tumors in the current agar culture system (for review see [21]). However, OV-CA represents a tumor type highly optimized for growth in the agar culture system [16]. Without question, agar culture represents an important new method for cytogenetic study of OV-CA.

5. Cytogenetic analysis of ovarian carcinoma: general observations

As previously mentioned, a large body of data exists on the cytogenetic analysis of human OV-CA. However, the majority of these studies have been performed prior to the advent of modern chromosome banding techniques, and therefore are of limited value. Despite the lack of banding analysis, certain general features of ovarian cancer which were first recognized in these early studies have been corroborated by more recent studies using

chromosome banding techniques. Comparing old and new data on OV-CA, the most common chromosomal pattern observed in OV-CA is aneuploidy (including both hyper- and hypodiploidy and numerous structural chromosome alterations). As a result of the substantial number of chromosome alterations observed in virtually all ovarian tumors (regardless of treatment history), a prominent feature of this cancer then is marked karyotypic variability. Characteristically, chromosome changes in OV-CA are reflected by modal chromosome numbers in the extreme hypodiploid (<40 chromosomes) or triploid-tetraploid range (~69–100 chromosomes), the presence of a large number of chromosomal markers (abnormal chromosomes with a peculiar morphology making them unique from all normal chromosomes), and the occasional presence of cells with substantial spontaneous chromosomal breakage.

5.1. Aneuploidy in ovarian carcinomas

Several studies have demonstrated a discontinuous nature of ovarian tumor samples showing a break in the hypo-hyperdiploid range. In early studies by Atkin [6], approximately 70% (17/23) of all OV-CA samples studied for chromosome number evidenced hyperdiploid DNA values. The remaining six low polidy cases were significantly hypodiploid, a finding somewhat at variance with other gynecologic tumors (e.g., cervix, endometrium [6]). This finding of a relatively frequent hypodiploid chromosome number in ovarian tumors is consistent with my laboratories experience in which approximately 10% of all ovarian tumors have demonstrated significant hypodiploidy. In our series of ovarian patients, those evidencing extreme hypodiploidy with modal chromosome numbers of $\ll 40$ have consistently demonstrated an extremely short survival duration [22]. This finding is particularly apparent in those patients with near-haploid chromosome numbers. Although the biologic explanation is unclear, in OV-CA as well as all other tumor types examined [23], near-haploid tumors have been associated with an extremely aggressive clinical course, resistance to standard treatment, and a short survival duration.

5.2. Structural chromosomal alterations in OV-CA

In early reports as well as more recent studies employing chromosome banding, numerous structurally altered 'marker' chromosomes have characterized OV-CA. The number of structurally altered morphologically recognizable markers varies greatly between patients. In my own laboratory experience, markers have ranged from as few as one per cell to as many as

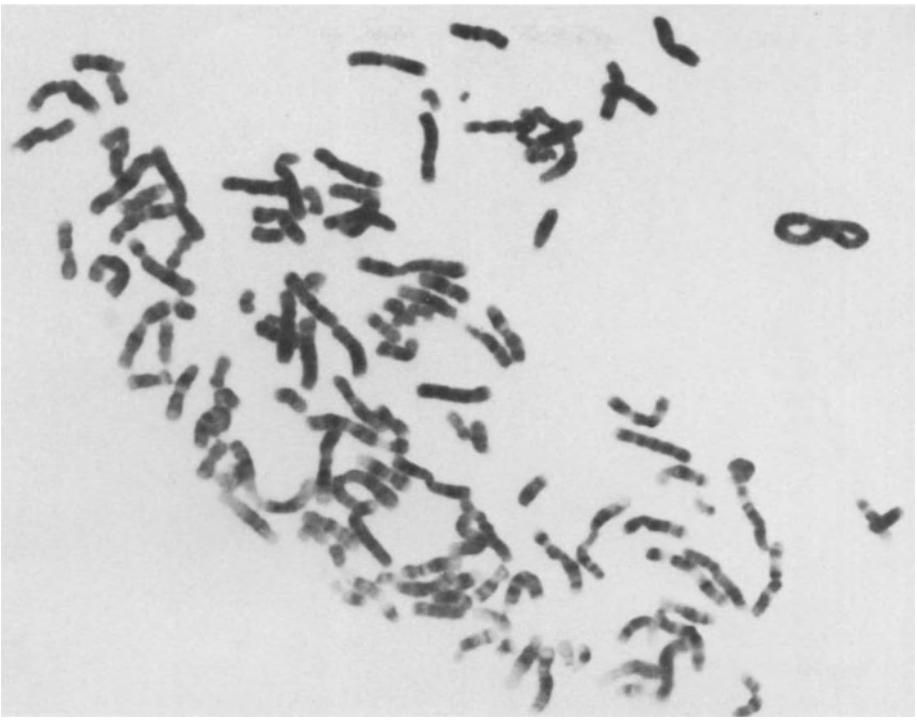

Figure 1. G-banded near-tetraploid metaphase cell from an untreated patient with OV-CA. Arrows point to numerous structurally altered abnormal chromosome 'markers'. Both numeric alteration of normal chromosomes, as well as formation of numerous marker chromosomes are common observations in OV-CA (see text).

several hundred in highly aberrant hexaploid cells. Interestingly, the percentage of structurally altered chromosomes bears no direct relationship to the ploidy level. Therefore, some near-diploid tumors may often contain virtually complete replacement of the normal chromosome set with abnormal markers, while some hyperdiploid tumors may often contain only exact multiples of normal chromosomes with few abnormal markers. An example of the bizarre nature of some of the marker chromosomes observed in ovarian tumors is demonstrated in Figures 1 and 2. As observed in these figures, rings, dicentrics, and other aberrent chromosome structures can almost entirely replace the normal complement. Early reports of OV-CA often noted the observation of extremely large markers, perhaps 1.5 times the size of chromosome 1 (the largest human chromosome) [1]. The apparent consequence of these long markers can often be observed in histologic section of interphase nuclei where protruding nuclear blebs often appear, as well as heavily stained chromocenters (Figure 3). As previously mentioned, the occurrence of these bizarre markers is not a reflection of ploidy level as both hypo and hyperdiploid cells may contain such alterations.

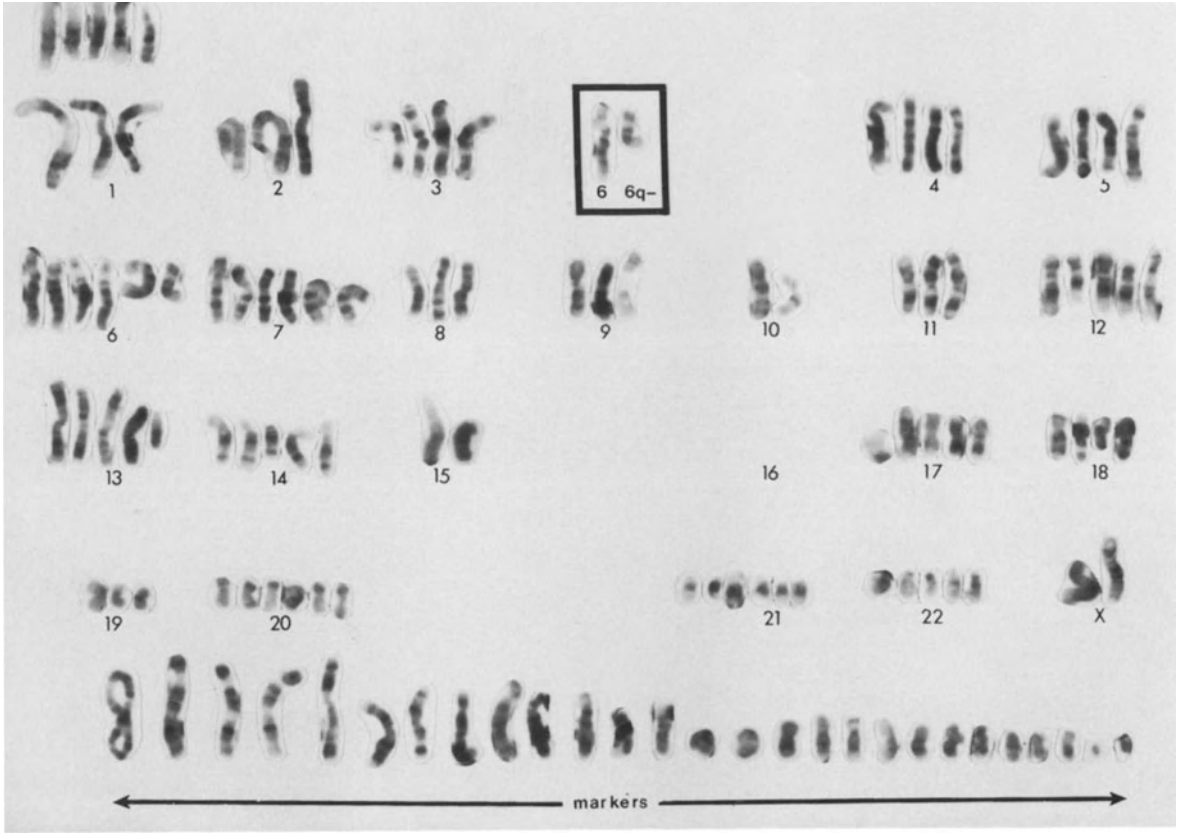

Figure 2. Karyotype of cell from Figure 1. Note the large number of marker chromosomes (bottom row). Solid box documents finding of a deletion of a portion of the long arm of chromosome 6 in this patient. Numerous alterations of chromosome 1 are among the significant number of chromosome changes in this tumor. Structural alterations involving recognizable segments of human chromosomes have been placed next to normal homologues for comparison (e.g. chromosome 13).

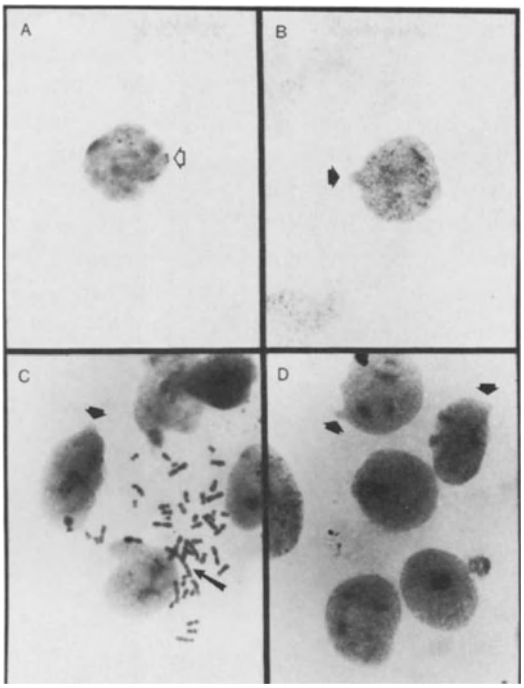

Figure 3. Example of nuclear protrusions in OV-CA caused by presence of long abnormal marker chromosomes. (a) G-banded nucleus showing heavily stained chromocenter (open arrow); (B, C, D) nuclei showing small nuclear protrusions (solid arrows); (C) tumor cell cluster with a G-banded metaphase with giant marker (thin arrow) and interphase nuclei nuclei with small protrusions (thick arrow) [from ref. 3 with permission].

The finding of frequent and extensive chromosomal rearrangements in OV-CA is somewhat at variance with other gynecologic tumors. Specifically, in banding analysis of cancers of the endometrium or cervix, chromosome numbers often closer to diploid are observed with banding analysis often demonstrating a relatively normal karyotype.

Because aneuploidy is such a characteristic feature of OV-CA studies, we have attempted to determine whether a correlation exists between the degree of chromosome change, and clinical treatment response or patient survival duration. As will be further described in section VII, preliminary results from our laboratory suggest that survival is inversely related to the level of chromosome change.

5.3. Spontaneous chromosome breakage in ovarian carcinoma

One additional and interesting relationship between the karyotypic findings and the clinical course of patients with OV-CA is found in patients with

extensive spontaneous chromosome breakage. In this small subset of patients (< 5 %) substantial chromosome breakage (chromosome breaks, gaps, quadraradials) are observed in virtually all cells from a patient's tumor (Figure 4, 5). This extensive type of chromosomal 'damage' is similar to that observed when cells are exposed to chemotherapy, radiotherapy, or other potent clastogenic (chromosome damaging) agents. However, the extensive chromosome breakage in these OV-CA patients is found *prior* to the advent of any clinical therapy. Reports from several different laboratories around the world have anecdotally noted the incidence of substantial breakage in direct preparations from a subset of patients with OV-CA [22, 24–

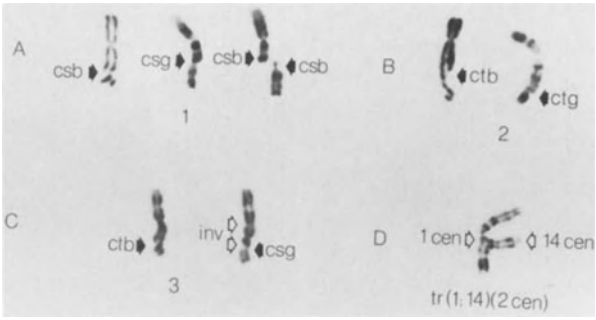

Figure 4A. Examples of extensive chromosome breakage observed in tumor cells from a patient with untreated ovarian adenocarcinoma (ref. 22). A) Examples of chromosome 1 breaks (csb) and gaps (csg): B) chromatid gaps (ctg) and breaks (ctb) from chromosome 2; inversion (inv) and breakage of chromosome 3; D) very unusual triradial formation between chromosomes 1 and 14 with maintenance of 2 centromeres (cen).

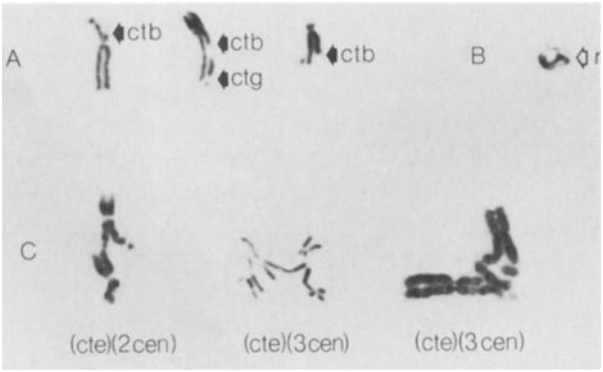

Figure 4b. Further examples of complex chromosome alterations involving multiple chromosomes: A) chromatid breaks and gaps; B) ring (r) chromosome; C) complex chromatid exchange (cte) with maintenance of up to 3 centromeres [from ref. 22 with permission].

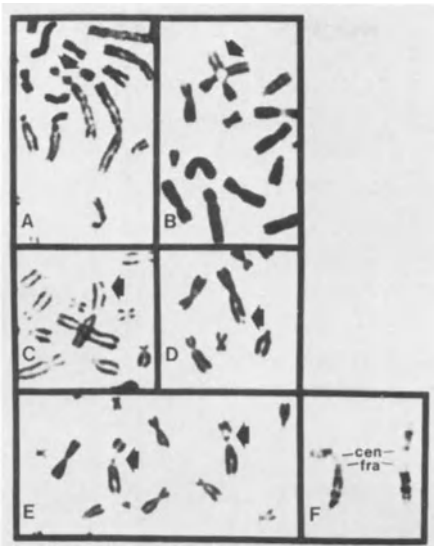

Figure 5. Example of chromosome alterations in peripheral blood lymphocyte (PBL) chromosomes from the patient studies in Figures 4A & 4B. A-E) Documentation of chromosome breaks (A, E), Gaps (C, D), and quadriradial formation (B). A previously documented 'fragile site' at chromosome 2q11 was also observed in PBL's from this patient (27, 28) [from ref. 22 with permission].

26. As is the case with patients displaying a near-haploid chromosome number, the clinical history of patients with substantial chromosome breakage is invariably extremely aggressive, with wide spread drug cross resistance, and extremely short survival duration.

Our findings [22] as well as previous work of others [24–26], clearly suggests that in those OV-CA patients displaying extensive spontaneous chromosome breakage prior to the initiation of therapy, the clinical history will invariably be dismal. We have speculated that the poor prognosis in patients with spontaneous chromosome breakage could be due in part to the rapid generation of intratumor karyotypic heterogeneity [23]. Thus, the manifestation of breakage of tumor chromosomal DNA, particularly to the degree expressed in the patient described in Figure 4, could be expected to lead to the rapid production of multiple stem-lines within a tumor providing a genetic basis for resistance to standard therapy. Specifically, following the clonal expansion in these patients' tumors, the eradication of all existing clones by any single (or often multiple) chemotherapeutic agent would appear unlikely. Certainly further investigation of chromosomal breakage in human cancers may provide important insights on genetic mechanisms of tumor progression and drug resistance in neoplasia.

6. Banded cytogenetic analysis of ovarian cancer

A review of the literature using modern banding techniques has demonstrated two major areas of consistent chromosome change in OV-CA, alterations of chromosomes 1 and 6. Each of these will be discussed separately below. In addition to the marked frequency of chromosome 1 and 6 alterations in this disease, marker chromosomes involving 3, and the precense of multiple double minutes (DMs) have frequently been reported in OV-CA [1, 3, 4]. During the discussions to follow on tumor associated chromosome change in OV-CA, it is important to recognize that these chromosomal alterations virtually always exist in the presence of further aneuploidy within the tumor genome. Thus, in large measure, the exact biochemical and biological effects of these alterations is currently indeterminate. However, as described in Section VIII, significant evidence now exists suggesting some tumor associated chromosome alterations may be associated with the resident site of human cellular oncogenes.

6.1. Chromosome 1 alterations in ovarian cancer

Chromosome 1 represents the most prevalent site of aberration in OV-CA, with ~80% of all tumors displaying either duplication, translocation, or inversions of # 1 (Figure 1). In a recent study by Whang-Peng *et al.* [4], chromosome 1 (and especially the short arm, 1p) was again shown to be the most frequent sight of chromosome alterations in this disease. Frequent change in the long arm of chromosome 1 (especially bands q21 → 44) are also extremely frequent sites of alterations in OV-CA. However, despite the prevalence of # 1 changes, these alterations are by no means 'specific' to ovarian cancers. Chromosome 1 alterations are extremely frequent in virtually all human carcinomas, and many hematopoietic malignancies [1]. One explanation put forth for the high frequency of chromosome 1 alterations in OV-CA (as well as other tumors) is a possible role in tumor progression (rather than initiation) for this chromosome change. Although this hypothesis has not yet been experimentally verified, it could help explain the extremely wide-spread occurrence of chromosome 1 alterations across a variety of histopathologic tumor types.

6.2. Chromosome 6 alterations in ovarian cancer

As mentioned previously, most cytogenetic studies prior to the development of chromosome banding (as well as several recent studies using banding techniques) have failed to demonstrated any 'specific' cytogenetic alter-

ations in OV-CA. However, results from our laboratory first presented in 1979 and later corroborated by numerous laboratories suggested the presence of a tumor 'associated' chromosome change in OV-CA: deletion or translocation involving a region of the long arm of chromosome 6 (bands q15–21) [Figure 1B] [3, 5]. Wake *et al.* in 1980 also described the common alteration of chromosome 6 in ovarian tumors and went so far as to suggest that a specific translocation of 6q [t(6;14)(q15;q24)] was involved with a specific histopathologic subtype of OV-CA (papillary serouscyst adenocarcinoma (OPSA) [5]). In this study, of 9 patients with OPSA, 5 tumors evidenced this apparently specific translocation with the remaining 4 tumors demonstrating either a 6q− or 14q+ chromosome. Although the specificity of the t(6;14) chromosome to OPSA is still to be independently corroborated, the importance of this study, we believe, was their finding that chromosome 6q alterations were again shown to be clustered at band regions q15→21. These results clearly suggest a possible role for chromosome 6q alterations in OPSA, while our studies appeared to extend the recognition of chromosome 6 abnormalities to ovarian cancers of different histologies. Subsequent studies by Kysuk *et al.* [29] and Woods *et al.* [30] have also demonstrated a common alteration of chromosome 6q in OV-CA. Recently, Whang-Peng *et al.* [4] performed cytogenetic analysis on malignant ascities or pleural fuilds from 44 patients with ovarian cancer. The results of their study suggested that chromosome 6q alterations were not 'specific' for ovarian cancer, that is, not every OV-CA studied contained a 6q−. However, the report of Whang-Peng *et al.* clearly supports our proposition that chromosome 6 alterations are indeed frequently 'associated' with this disease. Specifically, in their report, approximately 40% of all patients examined demonstrated chromosome 6 alterations with 70% of these patients evidencing a deletion of the long arm of chromosome 6. The difference between my laboratories study [3] and that of Whang-Peng *et al.* [4] appears to rest mainly in the use of the term 'specific' for the 6q chromosome change. This apparent controversy is unfortunate, as we have repeatedly pointed out that chromosome 6 alterations in ovarian cancer are not found in all cases:

> 'It is important to point out that a 6q− change is not specific only to ovarian neoplasms as this deletion has been reported in several other histologic types of cancers. Other cancers manifesting a loss of 6q are lung [31], cervix [31], melanoma [32], testicular cancer [33], and a wide variety of hematopoietic cancers (for review see [1])' [ref. 3].

The conclusion made by Whang-Peng *et al.*, in examining their own data on chromosome 6 change was that 'this marker (6q−) may be an artifact produced by *in vitro* selection in the agar feder layer procedure.' However, because only a small percentage of the total literature on OV-CA has been

obtained through the use of agar culture (which interestingly *does not* utilize a feder layer), and because all OV-CA's with 6q– markers published from our laboratory were examined by both agar and 'direct' cytogenetic techniques [3], this interpretation appears to be incorrect. Although chromosome 6 alterations are indeed not 'specific' (i.e., restricted to) ovarian neoplasms, there is a strong association between the alteration of 6q 15–23 and OV-CA. Of possibly great importance to this apparent controversy was the recent report by Harper *et al.* [34] providing a possible molecular correlate for the observed chromosome change on 6q. Specifically, the human cellular oncogene c-*myb* has recently been mapped by *in situ* hybridization to chromosome 6q 15–21, *the identical region of chromosome 6 we suggested was associated with OV-CA.*

In summary, the majority of studies of both primary and metastatic OV-CA as well as studies of numerous established OV-CA cell lines suggests that chromosome 6q alterations are a frequent feature of this disease. Although 6q alterations do not represent a 'specific' change (in a manner analogous to the Ph^1-chromosome in CML), their marked frequency in this disorder, along with a possible link to a human oncogenic sequence suggests that this chromosome change may play a biologically important role in the pathogenesis of OV-CA.

1. Drug resistance and genetic heterogeneity in human ovarian tumors

The term 'neoplastic progression' as first applied by Foulds [35] describes the process of divergence in biological characteristics of tumor cells, a process now widely believed to play a major role in determining clinical response at diagnosis. The generation of this tumor diversity has been hypothesized to occur via a stepwise selection of variant clones which have attained growth advantage through increasing genetic alterations [36]. Direct measurement of cellular alterations accompanying tumor progression are well defined in animal systems. Measurements of invasion and metastasis, loss of differentiated cellular characteristics, and increased evidence of chromosomal abnormalities have all been demonstrated to accompany increasing tumor progression in animal models [37–39]. Although much progress has been made in defining the cellular alterations in animal tumors, much less is known of the process of tumor progression in human cancers. We have utilized cytogenetic analysis of human tumor clonogenic populations to identify chromosomal alterations associated with time and/or treatment. Results of our preliminary studies in the context of previous work on tumor progression is detailed below.

Perhaps the strongest evidence for cellular changes associated with pro-

gression in human malignancies is provided by analysis of stem-line proportions through serial karyotypic studies of hematopoietic neoplasms [40]. Analysis of human hematopoietic cancers often demonstrate the appearance of secondary chromosomal changes superimposed onto an exsiting abnormal stem-line with progression of disease [40]. However, comparable studies relating karyotypic alteration to tumor progression in human carcinomas are extremely limited. Further, when reports of karyotypic evolution in carcinomas have appeared, they ordinarily have been complicated by simultaneous clinical treatment (i.e. chemotherapy or radiotherapy) during the cytogenetic sampling period [40, 41]. Recently, reports of progressive chromosome change in human carcinomas have appeared for one case each of malignant melanoma, ovarian carcinoma, and transitional cell carcinoma of the bladder [42–44]. These studies strongly suggest that progressive chromosomal evolution (i.e., increased number of markers, change in ploidy, etc.), may be associated with parallel increase in aggressiveness of disease. When these studies are viewed in context of recent cytogenetic analyses of animal tumors by Fidler and colleagues [45] and isoenzyme studies of Woodruff *et al.* [46], it appears that the development of many cancers may be contingent upon the clonal emergence of transformed cells bearing cytologic recognizable alterations. It is possible that associated with the process of tumor progression, introduction of a state of 'genetic plasticity' may occur, resulting in rapid production of clonal variants arising through step-wise selection of clones with growth advantage (e.g. the tumor progression model of Nowell [36], or as recently reported by Ling and colleagues, via stochastic generation of variants in high frequency [47]).

As mentioned previously, our laboratory has recently developed a method to cytogenetically analyze the clonogenic population of human carcinomas [3]. This technique has allowed the unequivocal identification of both inter- and intra-tumor karyotypic heterogeneity in a variety of human cancers including OV-CA. These studies have recently been combined and correlated with procedures described by Buick and colleagues which allow for analysis of cellular heterogeneity within human ovarian carcinoma cell populations [48–50]. The diversity of tumor cell populations present in these cancers is then demonstrated through karyotypic analysis of clonal proliferation in semi-solid media, analysis of cellular proliferation features (labelling index, clonogeneicity in soft agar, clonogenic cell self-renewal potential), cell differentiation features (histochemical differentiation, expression of ovarian differentiation antigens) and cell physical parameters (density/volume) [49]. Utilization of these different technologies has allowed us to examine in detail the appearance of tumor progression in human carcinomas by measuring cellular features in association with parallel chromosome change (49, and unpublished results). Our initial results support the findings from animal models, suggesting most tumors are indeed heterogeneous, and

tumor progression appears to arise from selection of pre-existing clonal variants [51, and unpublished data].

One explanation for treatment failure with cancer chemotherapy is the aforementioned tumor cell heterogeneity and its possible association with drug resistance within the tumor stem cell population at diagnosis. Currently, clinical recognition of most human cancers occurs at a tumor burden of $\geq 10^8$ cells. This cell number is well beyond the mutation rate of many drug resistant mutants, as measured in cultured mammalian cell lines (i.e. $\sim 10^6$ [52]). These *in vitro* estimates of mutation rate (derived from study of established cell lines) have served as the basis for the Goldie-Coldman hypothesis [53] which relates drug resistance of tumor stem cells to tumor burden, and spontaneous mutation rate. It is essential to this model that accurate measurement of the *in vivo* mutation rate be achieved. As recently pointed out by Ling in his review of the genetic basis of drug-resistance:

> 'In the context of our current understanding that genetic instability and karyotypic diversity are intimately associated with malignant transformation and tumor progression, it seems entirely possible that mutation to drug resistance could arise in spontaneous tumors at a rate much higher than previously anticipated [52],'

Based upon our karyotypic analysis of human solid tumors, we have suggested that single loci mutation rates (derived from stochastic generation of drug resistant phenotypes) may, in fact, underestimate the mutation rate of spontaneous human tumors which exhibit significant clonal karyotypic heterogeneity [54]. It is therefore possible that a direct relationship exists between increasing chromosome change and the presence of drug resistant populations. In fact, very preliminary results suggest that as the degree of chromosomal alterations increases the survival duration decreases, with a corresponding increase in relative drug resistance [54]. Based upon our preliminary data, it appears possible that estimation of karyotypic heterogeneity may prove a therapeutically significant variable of high relevance in defining drug resistant phenotypes.

In summary, progression of malignant features in OV-CA can be characterized by a variety of laboratory techniques. Preliminary cytogenetic data from our laboratory [54] and others [4, 6, 7] suggests that the degree of chromosome alteration may provide a biologically relevant endpoint to relate to a patient's clinical response to therapy, and ultimately to patient survival.

8. The association of oncogenes with chromosome abnormalities in human cancer

A remarkable concordance has recently been observed between the chromosomal breakpoint sites common to certain human tumors and the chro-

mosomal site of certain human cellular oncogenes (c-*onc* genes) [34, 55, 56]. This finding is especially significant with the recent discoveries that several transforming retrovirus (v-*onc* genes) share DNA sequence homology to c-*onc* genes (for review see [57]). Although the exact biological function of the c-*onc* genes in the normal biology of vertebrate cells is unclear, several recent studies have linked c-*onc* genes to cellular 'growth factors', important agents in initiating cell division and transformation. Perhaps the most significant findings so far in regard to the possible functional significance of a c-*onc* gene was the determination that the v-*erb* B and c-*sis* oncogenes contain DNA sequences homologous to epidermal growth factor (EGF) [58] and platelet derived growth factor (PDGF) [59] respectively. These findings appear to provide strong support for models of oncogenesis that suggests a specific alteration in the expression of a 'normal' cellular gene (e.g., c-*onc* gene) may lead to expression of the transformed or malignant state [60].

Among the leading candidates for inducing altered expression of a c-*onc* gene is chromosomal alteration. The best described study to date linking a specific cytogenetic change with a specific functional c-*onc* gene is the analysis of B-cell neoplasmas and the human cellular oncogene c-*myc*. Recent studies of these cancers has revealed a remarkable concordance between the sites of immunoglobulin genes and the sites of specific chromosomal translocation. Thus, chromosome mapping of the immunoglobulin genes (present on four specific chromosomal loci) are now known to be the exact chromosomal regions commonly altered in B-cell cancers. The breakthrough in this research came recently when Dalla-Favera *et al.* [55] and Taub and Leder [56] demonstrated that the cellular oncogene c-*myc* (the cellular counterpart of the transforming MC29 virus) resides on chromosome 8q24; *the same site specifically altered in approximately 90% of all patients with Burkitt's lymphoma.* These results appear to link the chromosomal site of the c-*myc* oncogene with an immunoglobulin coding sequence, and may further tie together mechanisms of antibody gene diversity with mechanisms of carcinogenesis. Importantly, a similar observation between the chromosomal site of a c-*onc* gene and a recognized site of tumor associated chromosome change has been documented for a variety of cancers (for review see [61]).

With specific reference to OV-CA, the possibility exists that two different oncogenes (c-*ras* and c-*myb*) may be associated with this disease. Specifically, members of the c-*ras* gene family have recently been localized to chromosome 1 and 3 [61], chromosomes often altered in OV-CA. Further, as mentioned previously, c-*myb* oncogene has recently been mapped to a region frequently altered in OV-CA (chromosome 6q 15–21) [34] (see section 6.2). These findings (when combined with those from other neoplasmas) have lead several investigators to examine c-*ras* and c-*myb* expression in OV-CA. The results of a very recent study using an NIH-3T3 cell trans-

fection assay have indicated that at least some ovarian cancers express an altered c-*ras* oncogene [62]. Further, preliminary evidence suggests c-*myb* is overexpressed in some cases of OV-CA (Personal Communications, Stephen Howell, Department of Oncology, University of California, San Diego).

The association of c-*onc* genes with the site of specific chromosomal alterations provides a possibly important 'trigger' for inducing the transformed state. The potential for our achieving therapeutic benefit from the insights into the basic molecular mechanisms of oncogenesis previously discussed appears enormous. It is possible that within the next decade discoveries now being made on the molecular level will translate into completely novel treatment modalities. Understanding of how these basic genetic mechanisms associate with chromosome change are only now providing us with our first glimpses into what is believed to be the actùal origins of human cancers. The promise of a tremendous increase in our knowledge of human cancers appears within our grasp. Without doubt, analysis of the molecular and cytogenetic features of OV-CA will continue to receive increasing attention in the upcoming years.

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References

1. Sandberg AA: The chromosomes in human cancer and leukeima. Elsevier, 1980.
2. Curcio S: Analisi cromosomica di un folliculoma, Estratto dagli atti del III Congresso della Societa Italiana di Citologia Clinica et Sociale. Arch Ostet Ginecol 71:139–143, 1966.
3. Trent J, Salmon SE: Karyotypic analysis of human ovarian carcinoma cells cloned in short term agar culture. Cancer Genetics & Cytogenetics 3:279–291, 1981.
4. Whang-Peng J, Knutsen T, Douglass EC, Chu E, Ozols RF, Hogan WM, Young RC: Cytogenetic Studies in Ovarian Cancer. Cancer Genetics and Cytogenetics 11:91–106, 1984.
5. Wake N, Hreshchyshyn MM, Piver SM, Matsui S, Sandberg AA: Specific cytogenetic changes in ovarian cancer involving chromosomes 6 and 14. Cancer Res 40:4512–4518, 1980.
6. Atkin NB: Modal DNA value and chromosome number in ovarian neoplasia. A clinical and histopathologic assessment. Cancer 27:1064–1073, 1971.
7. Atkin NB: Cytogenetic studies on human tumors and premalignant lesions: The emergence of aneuploid cell lines and their relationship to the process of malignant transformation in

man. In: Genetic Concepts and Neoplasia, (ed) by Staff of the Univ of Texas and MD Anderson Hospital, 23rd Annual Symposium on Fundamental Cancer Research, University of Texas MD Anderson Hospital Tumor Institute, Houston, Texas, 1969, pp 36–56. Baltimore, MD: Williams & Wilkins, 1970.

8. Kakati S, Hayata I, Oshimura M, Sandberg A: Chromosomes and causation of human cancer and leukemia: X. Banding patterns in cancerous effusions. Cancer 36:1729–1738, 1975.
9. Shiloh Y, Cohen M: An improved technique of preparing bone marrow specimens for cytogenetic analysis. In Vitro 14:510–513, 1978.
10. Kakati S, Sandberg AA: Chromosomes in solid tumors. Virchows Arch B (Cell Pathol) 29:129–137, 1978.
11. Yunis JJ: New Chromosome techniques in the study of human neoplasia. Human Pathology 12:6–549, 1981.
12. Yunis JJ, Ramsey N: Retinoblastoma and subband deletion of chromosome 13. Am J Dis Child 132:161–163, 1978.
13. Wake N, Slocum H, Rustum Y, Matani S, Sandberg A: Chromosomes and causation of human cancer and leukemia XLIV. A method for chromosome analysis of solid tumors. Cancer Gene Cytogene 3:1–10, 1981.
14. Yunis JJ: Human chromosome methodology, 2nd edition, Academic Press Inc, New York, 1974.
15. Trent JM, Salmon SE: Human tumor karyology: Marked analytic improvement by short-term agar culture. Br J Cancer 41:867–874, 1980.
16. Hamburger AW, Salmon SE, Kim MB, Soehnlen BJ, Trent JM, Alberts DJ: Direct cloning of human ovarian carcinoma cells in agar. Cancer Res 38:3438–3444, 1978.
17. Trent JM: Protocols of procedures and techniques in chromosome analysis of tumor stem cell cultures in soft agar. In: Salmon SE (ed), Cloning of human tumor stem cells. Alan R. Liss, Inc, New York, 1980, pp 345–349.
18. Slocum HK, Pavelic ZP, Frankfurt O, Wake N, Sandberg AA, Greco WR, Rustum YM: Clonogenic assay and other parameter measurements of cells from human lung carcinoma and other solid tumors, 3rd NCI-EORTC symposium on new drugs in cancer therapy, Brussels, Belgium (meeting abstract), 1981.
19. Dow LW, Bhakta M, Williams J: Clonogenic assay for Wilm's tumor: improved technique for obtaining single-cell suspensions and evidence for tumor cell specificity. Cancer Res 42:5262–5264, 1982.
20. Carney DN, Gazdar AF, Bunn PA, Guccion J: Demonstration of the stem cell nature of clonogenic tumor cells from lung cancer patients. Stem Cells 1:149–164, 1981.
21. Salmon SE: Perspectives in the HTSCA. In: Human tumor cloning, Salmon SE, Trent JM (eds), Grune & Stratton, New York, NY (in press 1984).
22. Trent JM: Spontaneous chromosome breakage in ovarian adenocarcinoma. Cancer Gene & Cytogene. (in press 1984).
23. Trent JM, Stanisic T, Olson S: Cytogenetic analysis of urologic malignancies: Study of tumor colony forming cells and premature chromosome condensation. J of Urology 131:146–151, 1984.
24. Atkin NB, Baker MC, Brandao HJS: Chromosomes in effusions. Lancet ii, 1367, 1967.
25. Fraccaro M, Gerli M. Tiepolo L, Zara C: Analisi della variabilita cariotipica in un caso de neoplasia ovarica. Minerga Ginecol 17:485–492, 1965.
26. Zara C, Fraccaro M, Gerli A, Mannini A, Tiepolo L: L'effetto del trattamento con ciclofosfamide sulla dinamica cromosomica di popolazioni di cellule tumorali in vivo. Clin Obstet Ginecol 71:(suppl) 103, 1966.
27. Sutherland GR: Heritable fragile sites on human chromosomes I. Effects of composition of culture medium on expression. Am J Hum Gen 31:125–135, 1979.

28. Sutherland GR: Heritable fragile sites on human chromosomes II. Distribution, phenotypic effects, and cytogenetics. Am J Hum Gene 31:136–148, 1979.
29. Kusyk CJ, Seski JC, Medlin WV, Edwards CL: Progressive chromosome changes associated with different sites of one ovarian carcinoma. J Natl Cancer Inst 66:1021–1025, 1981.
30. Woods LK, Morgan RT, Quinn LA, Moore GE, Semptle TU, Stedman KE: Comparison of four new cell lines from patients with adenocarcinoma of the ovary. Cancer Res 39:4449–4459, 1979.
31. van der Reit-Fox M, Retief A, Nierkerk W: Chromosome changes in 17 human neoplasms studied with banding. Cancer 44:2108–2119, 1979.
32. Kakati S, Song S, Sandberg A: Chromosomes and causation of human cancer and leukemia: XXII. Karyotypic changes in malignant melanoma. Cancer 40:1173–1181, 1977.
33. Bronson D, Andrews P, Solter D, Cervenka J, Lange P, Fraley EE: Cell line derived from metastasis of a human testicular germ cell tumor. Cancer Res 40:2500–2506, 1980.
34. Harper M, Franchini G, Lane J, Simon MI, Gallo RC, Wong-Staal F: Chromosomal sublocalization of human c-myb and c-fes cellular onc genes. Nature 304:169–171, 1983.
35. Foulds L: Neoplastic Development, Vol. I, Academic Press, New York, 1969.
36. Nowell PC: Clonal evolution of tumor cell populations. Science 194:23, 1976.
37. Gray JM, Pierce GB: Relationships between growth rate and differentiation of melanoma *in vivo*. J Natl Can Inst 32:1201, 1964.
38. Kiane DT, King M, Zhang HJ, Kennedy BJ, Wang N: Cyclic biologic expression in mouse mammary tumors. Science 216:68, 1982.
39. Kline G, Kline E: Conversion of solid neoplasms into ascites tumors. Ann New York Acad Science 63:640, 1956.
40. Sandberg AA, Ichara T, Moore GE, Pichren JW: Unusually high polyploidy in a human cancer. Cancer 16:1246, 1963.
41. Sandberg AA, Yamada K: Chromosomes and causation of human cancer and leukemia I. Karyotypic diversity in a single cancer. Cancer 19:1869, 1966.
42. Balaban G, Herlyn M, Guerry D, Nowell P: Karyotype stability in the primary tumor and multiple metastases of a human melanoma. Proc Am Assoc Cancer Res 23:130a, 1982.
43. Falor WH, Ward RM: Fifty-three month persistance of ring chromosome in non-invasive bladder carcinoma. Acta Cytol 20:272, 1976.
44. Kusyk CJ, Edward CL, Arrighi FE, Romsdahl MM: Progressive chromosome change associated with different sites of one ovarian carcinoma. J Nat Can Inst 65:1199, 1979.
45. Talmadge JE, Wolman SR, Fidler IJ: Evidence for the clonal origin of spontaneous metastases. Science 217:361, 1982.
46. Woodruff MFA, Ansell JD, Forbes GM, Gordon JC, Burton DI, Micklem HS: Clonal interactions in tumors. Nature 299:822, 1982.
47. Harris JF, Chambers AF, Kill RP, Ling V: Metastatic varients are generated spontaneously at a high rate in mouse KHT tumor. Proc Natl Acad Sci 79:5547, 1982.
48. Buick RN, Mackillop WJ: Measurement of self-renewal in culture of clonogenic cells from human ovarian carcinoma. Br J Cancer 44:349, 1980.
49. Mackillop WJ, Buick RN: Cellular heterogeneity in human ovarian carcinoma studied by density gradient fractionation. Stem Cells 1:355, 1982.
50. Mackillop WJ, Trent JM, Stewart SS, Buick RN: Tumor progression studied by analysis of cullular features of serial ovarian carcinoma ascites. Cancer Res 43:874, 1983.
51. Trent JM, Thompson FH, Buick RN: Generation of clonal varients in a human ovarian carcinoma studied by chromosome banding analysis. Cancer Gene. and Cytogene 14:153–161, 1984.
52. Ling V: Genetic Basis of drug resistance in mammalian cells. In: Drug and Hormone Resistance in Neoplasia. Bruchovsky N, Goldie J (eds), Press, New York, p 14.
53. Goldie JH, Coldman AJ: A mathematical model for relating the drug sensitivity of tumors

to the spontaneous mutation rate. Cancer Treatment Reports 63:1727, 1979.
54. Trent JM, Salmon SE, Thompson FH, Buick RN: Drug resistance and genetic heterogeneity in spontaneous human tumors. 13th International Vienna Congress of Chemotherapy, Volume 11, #224, pp. 38–42, 1983.
55. Dalla-Favera R, Bergni M, Erikson J, Patterson D, Gallo RC, Croce CM: Human c.*myc onc* gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. Proc Natl Acad Sci USA 79:7824–7827, 1982.
56. Taub R, Kirsch I, Morton C, Lenoir G, Swan D, Tronick S, Aaronson S, Leder P: Translocation of the c-*myc* gene into the immunoglobulin heavy chain locus in human Burkitt's lymphoma and murine plasmacytoma cells, Proc Natl Acad Sci USA 79:7837–7841, 1982.
57. Bishop JM: Cellular oncogenes. In: Ann Review Biochem, Annual Reviews Inc, Palo Alto, California, 1983.
58. Downward J, Yarden Y, Mayes E, Scrace G, *et al.*: Close similarity of epidermal growth factor receptor and v-*erb* B oncogene protein sequences. Nature 307:521–526, 1984.
59. Waterfield MD *et al.*: Platelet-derived growth factor is structurally related to the putative transforming protain P 28 sis of simiam sarcoma virus. Nature 304:35–39, 1983.
60. Klein G: The role of gene dosage and genetic transpositions in carcinogenesis. Nature 294:313–318, 1981.
61. Le Beau M, Rowley J: Heratible fragile sites in cancer. Nature 308:607, 1984.
62. Feig LA, Bast RC, Knapp RC, Cooper GM: Somatic activation of ras k gene in a human ovarian carcinoma. Science 223:698–701, 1984.

2. Recent advances in the immunodiagnosis of epithelial ovarian carcinoma

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Since we last reviewed the immunology of ovarian carcinoma for this series [1], there have been several advances in the development of serum markers for epithelial ovarian cancer. Much of this progress has related to the identification of tumor associated antigens using the monoclonal technology [2].

Markers defined by monoclonal antibodies

The murine monoclonal antibody OC 125 was raised against a serous cystadenocarcinoma [3]. The antibody binds to a determinant designated CA 125 which is associated with at least two mucin like molecules of greater than 200,000 daltons. Multiple CA 125 determinants are present on each of these molecules. CA 125 is associated with coelomic epithelium and amnion during embryonic development [4]. Traces of CA 125 are found in adult tissues derived from coelomic epithelium, including the pleura, peritoneum and pericardium, as well as the epithelial cells lining the fallopian tube, endometrium and endocervix [4]. CA 125 has been detected in endocervical mucus consistent with the physiological secretion of the antigen. Significantly, CA 125 is also associated with more than 80% of nonmucinous epithelial ovarian carcinomas [3, 5]. Preliminary data suggest that CA 125 is expressed both by clonogenic and by non-clonogenic tumor cells [6].

CA 125 is shed into the supernatants of cultures containing epithelial ovarian carcinoma cells [7]. A radioimmunoassay has been developed to detect shed antigenic determinants in serum and body fluids [8, 9]. Antigen activity has been defined in arbitrary units relative to a standard preparation from culture supernatants or ascites fluids. Using this assay, the smallest amount of antigen that can be measured reliably is 1.4 U/ml. The assay is linear from 1 to 500 U/ml. Following dilution, samples containing more than 20,000 U/ml can be assayed conveniently. The day to day coefficient of variation for the assay is approximately 15%. Consequently, a doubling or

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halving of antigen levels has been considered significant. When samples from 888 apparently healthy blood bank donors were assayed, only 1% had serum CA 125 values greater than 35 U/ml. Using the same limiting value, 6% of patients with benign disease and 28% of individuals with nongynecologic malignancies had elevated blood levels. Antigen levels were greater than 35 U/ml in more than 80% of patients with nonmucinous epithelial ovarian cancers. Increases or decreases in antigen levels paralleled progression or regression of disease in more than 90% of instances studied in the initial trial. In some cases, elevated CA 125 has been observed 3–7 months prior to disease recurrence [8, 10]. In confirmatory studies, CA 125 levels have been >30 U/ml in 92% [11], >35 U/ml in 78% [12] and >65 U/ml in 63% [13] of ovarian cancer patients entered in different studies. CA 125 levels correlated with diseases course in 83 to >90% of instances [11–13]. Elevations of CA 125 have preceeded disease recurrence by 1 to 6 months in 8 of 13 individuals [12].

CA 125 may also prove useful in monitoring other gynecological neoplasms which arise from Mullerian duct derivatives. Antigen levels have been elevated in 70% of patients with advanced endometrial carcinoma, carcinoma of the fallopian tube and adenocarcinoma of the endocervix [14]. With rare exceptions, elevations of antigen levels have not been observed in patients with squamous cell carcinoma of the cervix, vagina or vulva. It remains to be seen whether increases or decreases in antigen levels for these other tumors will parallel disease progression and regression.

Preliminary data suggest that CA 125 might prove useful for early detection of ovarian malignancy. In one fortuitous case, elevation of CA 125 preceeded the primary diagnosis of disease by 10 to 12 months [15]. Preoperative CA 125 values have exceeded 65 U/ml in sera from 8 of 11 patients with Stage I and Stage II epithelial ovarian cancer (Knapp *et al.*, unpublished data). When sera were studied from more than 1,000 patients attending a gynecology clinic, CA 125 levels were elevated in 16% of women in first trimester pregnancy [16]. CA 125 was found at high concentration in amniotic fluid consistent with its histochemical localization in amnion. In 988 nonpregnant patients with benign gynecologic disorders, CA 125 was >65 U/ml in 1% on a single determination and in 0.5% with two determinations [16]. Persistently elevated or progressively rising levels of CA 125 are likely to have greater predictive value than a single abnormal value. The critical issue for screening, however, is whether CA 125 can distinguish apparently healthy individuals with neoplastic and non-neoplastic diseases. Recent studies suggest that CA 125 levels can be elevated in cirrhosis [11, 17], particularly when it is associated with ascites formation [11]. Hepatocellular disease of this magnitude is generally apparent clinically and is reflected in abnormal liver function tests. Association of CA 125 with different gynecological and nongynecological cancers is not necessarily a

disadvantage, provided that the primary tumors from different sites can be distinguished by noninvasive techniques.

In contrast to ovarian cancer, CA 125 is not generally elevated in early stages of endometrial carcinoma [14]. This may reflect shedding of tumor associated antigen into the endometrial cavity, rather than into lymphatics or blood vessels. As had been discussed in our earlier review [1], the unique biology of ovarian cancer may favor the early appearance of tumor associated antigen in the peripheral circulation. Whether or not the CA 125 assay will be sufficiently sensitive to provide useful lead time for diagnosis in a significant fraction of ovarian cancer patients remains to be determined. If our preliminary observations regarding preoperative CA 125 levels are confirmed in larger numbers of patients with early stage epithelial ovarian cancer, a trial might be undertaken involving as many as 100,000 women age 40–70. During 100,000 woman-years of observation in the United States, 20–40 cases of epithelial ovarian cancer would be expected in this age group. Even if CA 125 does not prove to be a useful marker for screening, such a clinical trial would establish a serum bank that could be used to evaluate new diagnostic tests based on other monoclonal reagents within weeks rather than years of their development.

Since the development of OC 125, several monoclonal reagents have been described which react with epithelial ovarian cancers [18–23]. Several of these reagents bind to tumors with mucinous histology which fail to react with OC 125 [18, 19, 22]. 1D3 was obtained after immunization with an extract from an undifferentiated ovarian carcinoma and reacts with 12 of 14 mucinous cystadenocarcinomas, but not with tumors of serous or endometrioid histology [18]. Reactivity with extracts of fetal intestine has been detected in a radioimmunoassay, but here was no reactivity with carcinoembryonic antigen (CEA), normal glycoprotein (NGP), adult tissues or serum components.

MOV-2 is an IgM monoclonal that reacts with a carbohydrate determinant expressed on a high molecular weight glycoprotein which is found in mucinous glands of the digestive tract, larynx and exocrine pancreas as well as lactating mammary duct [19]. The antibody reacts with approximately 75% of well differentiated ovarian carcinomas, including tumors of mucinous histology. MOV-2 reacts with 22% of undifferentiated ovarian carcinomas as well as 40% of the nonovarian malignancies tested. By contrast, OC 133 which binds to adult endometrium and endocervix [20] appears to react with an 80,000 dalton moiety [7] expressed predominantly by tumors of serous morphology [20].

Recently, monoclonal reagents raised against ovarian and endometrial carcinoma cell lines have been used to characterize 5 additional antigens [21]. MD144, MF61 and MF116 antigens were all expressed by 1 of 8 ovarian tumor cell lines tested, but differed in reactivity with other cell lines

and tissues. MD144 appears to be a glycolipid that is only associated with the ovarian tumor cell line used to immunize the murine donor and not with 152 other cell lines. MF61 is also a glycolipid that is associated with non-cellular follicles of the thyroid, uterine glandular cells and 6 of 16 renal cell carcinoma cell lines. MF116 is an acidic glycoprotein of 105,000 daltons that is expressed by kidney epithelial cells as well as a fraction of cell lines established from neuroblastomas and endometrial, renal and bladder carcinomas. MH55 and MH94 are antigens recognized by antibodies raised against an endometrial carcinoma cell line which can be detected by mixed hemadsorption on 4 of 8 and 2 of 8 ovarian carcinoma cell lines respectively. MH94 is widely expressed on normal and malignant epithelial cells, but MH55 could not be detected in a large number of normal tissues and non-gynecological tumor cell lines [21]. Whether or not MH55 will provide a useful serum marker remains to be determined.

Several other monoclonal antibodies have been raised against nonovarian tumor tissues and subsequently found to react with epithelial ovarian cancers. DUPAN-2 was developed against a human pancreatic cancer cell line [22] and binds to ductal epithelial cells of the adult pancreas. Although the antibody binds to secretory cells of the fetal small intestine and salivary gland, DUPAN-2 does not bind to fetal pancreas. A radioimmunoassay has been developed to monitor the course of pancreatic carcinoma [23]. Sera from 80% of 54 patients tested had > 300 U/ml of antigen activity, whereas sera from each of 63 normal individuals had < 300 U/ml. Preliminary data suggest that the antigen can also be found in sera from a fraction of patients with ovarian carcinomas of serous, endometrioid and mucinous histology [24]. In several cases DUPAN-2 antigen levels have correlated with the clinical course of ovarian cancer patients. DUPAN-2 appears to be a carbohydrate determinant associated with a mucin-like glycoprotein of > 500,000 daltons that is found on molecules distinct from those which bear CA 125 determinants (R.S. Metzgar, personal communication). The F36/22 monoclonal antibody, originally raised against human breast cancer cells [25], also recognizes a glycoprotein of > 700,000 daltons that is associated with a majority of breast carcinomas [26] and 19 of 19 epithelial ovarian tumors, including examples from each of the major histological subtypes [27]. The F36/22 does not bind to mesothelial cells, but does react with ductular epithelium from the non-lactating adult breast which sets it apart from MOV-2, DUPAN-2 and OC 125. Reactivity with mucinous cystadenocarcinomas [29] further differentiates F36/22 from OC 125 [5].

The 19-9 antibody provides yet another example of a murine monoclonal reagent that binds to carbohydrate determinants expressed on a high molecular weight mucin-like molecule which can be associated with epithelial ovarian carcinomas. 19-9 is produced by a hybridoma that was prepared from spleen cells of mice immunized with a human colorectal cell line [28].

The antibody recognizes sialylated Lewis blood group A determinants which can be expressed either on cell membrane gangliosides or on a mucin-like glycoprotein of > 500,000 daltons molecular weight [29]. Antigenic determinants (designated CA 19-9) in the sera of cancer patients are associated predominantly with the mucin rather than with the ganglioside [29]. A solid-phase radioimmunometric sandwich assay has been developed to detect CA 19-9 in serum and body fluids [30]. Using this assay elevated CA 19-9 levels (> 37 U/ml) have been found in sera from a majority of patients with advanced pancreatic, gastric and colorectal carcinomas [31]. An immunohistochemical study which utilized the biotin-avidin immunoperoxidase technique detected CA 19-9 in 27% of serous, 40% of endometrioid, 57% of clear cell and 76% of mucinous ovarian tumors [32]. In the same study CEA was found in 62% of benign, borderline and malignant mucinous tumors. As CA 19-9 and CEA are associated with a subset of ovarian tumors that fail to express CA 125, all 3 markers were measured in a panel of sera from ovarian cancer patients to determine whether the concomitant measurement of CA 125, CA 19-9 and CEA would provide a more precise correlation with tumor burden for a larger fraction of ovarian cancer patients than could be obtained with any single assay [10]. Among 105 patients with surgically demonstrable ovarian carcinoma, serum CA 125 levels were elevated (> 35 U/ml) in 83%, CA 19-9 (> 37 U/ml) in 17% and CEA (> 2.5 mg/ml) in 37%. At least one of the three markers was elevated in 90% of the subjects. When 41 patients were monitored serially over 2 to 60 months, alteration in abnormal CA 125 levels correlated with disease progression or regression in 94% of instances, CA 19-9 in 33% and CEA in 25%. Given the high degree of correlation between CA 125 levels and clinical course, concomitant measurement of the 3 markers did not prove superior to the measurement of CA 125 alone. Few patients in this study, however, had tumors with a mucinous histology. Consequently, a possible contribution of CA 19-9 and CEA to monitoring deserves further evaluation in selected patients with elevated serum levels of these markers.

Within individual serum samples, no correlation was found among values for CA-125, CA 19-9 and CEA, but nearly all patients with elevated CA 19-9 levels also had increased levels of CA 125. In one patient all 3 markers were monitored on 29 occasions over an 8 month period [10]. An increase in CA 125 was observed at least 3 months before the earliest evidence of disease progression was obtained by noninvasive techniques. CA 19-9 levels paralleled those of CA 125, exhibiting a pattern that was qualitatively quite similar, but quantitatively less impressive than those obtained with CA 125. A significant increase in CA 19-9 was observed 2 months before evidence of disease progression. During the period of observation, CEA levels did not change significantly and remained within a normal range. Considering the parallel between CA 125 and CA 19-9, as well as the observation that ele-

vated CA 19-9 levels were almost always associated with elevated CA 125, we have examined the relationship between the two determinants on mucin-like molecules from the sera and ascites fluid of ovarian cancer patients.

Addition of excess 19-9 antibody did not inhibit measurement of CA 125 antigen in the CA 125 radioimmunoassay [33]. Conversely addition of excess OC 125 did not inhibit measurement of 19-9 antigen in the CA 19-9 assay [33]. Consequently, the CA 125 and CA 19-9 epitopes appear antigenically distinct. If, however, partially purified CA 125 antigen is electrophoresed and transblots are prepared with ^{125}I-OC 125 and ^{125}I-19-9 antibodies, two bands are observed at > 500,000 daltons. A very high molecular weight moiety binds both OC 125 and 19-9, whereas a moiety of somewhat lower molecular weight binds only OC 125 [33]. Sera from patients with elevated CA 125 levels were incubated with OC 125 on a solid phase immunoadsorbant and bound antigen was analyzed by subsequent incubation with ^{125}I-19-9. Sera from approximately 40% of patients with elevated levels of CA 125 had elevated levels of molecules which contained both CA 125 and CA 19-9 determinants [33]. One possible explanation is that both CA 19-9 and CA 125 are carbohydrate determinants that are transferred to large mucin-like molecules, either individually or in combination depending upon the aberrant transferase and glycosidase profiles of individual tumor cells.

Glycosyltransferases catalyze the addition of individual monosaccharides to oligosaccharides associated with glycoproteins and glycolipids [34]. Specificity is maintained both for the monosaccharide and for the acceptor molecule. Conversely, glycosidases catalyze the cleavage of monosaccharide residues from the same glycoconjugates. Abnormal expression of glycosyltransferases ad glycosidases has been observed in a number of different human neoplasms. In studies with ovarian tumor homogenates enzymatic activities of a variety of glycosyltransferases have been elevated when compared to the activities in homogenates prepared from normal ovary [34]. Although the latter may not be an entirely adequate control, considering the rather small contribution of normal surface epithelium to any homogenate of whole ovary, elevations of galactosyl-, sialyl- and fucosyl-transferases were found in more than 80% of tumor specimens tested [34]. When serum specimens were examined, galactosyl-, N-acetylglucosaminyl-1- and N-actylglucosaminyl-2-transferases were elevated in more than 80% of patients with surgically demonstrable epithelial ovarian tumors [34]. Subsequent studies have confirmed the presence of elevated galactosyl transferase levels in the serum of patients with ovarian carcinoma [35, 36]. Of 17 different biochemical and oncofetal markers evaluated in one study, galactosyl transferase appeared to be the most promising [35]. In the largest series reported to date, galactosyl transferase was elevated in 53% of 59 patients

with residual ovarian cancer, compared to 0% of 28 controls and 9% of 54 ovarian cancer patients who were thought to be free from disease [36]. Correlation was found between galactosyltransferase levels and clinical course in 18 of 34 patients (53%). In 5 additional patients galactosyl transferase levels rose after clinical recurrence of tumor had become evident [36].

Abnormalities of glycosidases have also been detected in ovarian tumor tissue [34]. N-acetyl-B-D-glycosaminidase, N-acetyl-B-D-galactosaminidase and N-acetyl-B-D-galactosidase were elevated in a majority of specimens tested. When glycosidase profiles were examined in greater detail, B-hexoseaminidase levels were elevated in poorly differentiated ovarian cancers, but not in well differentiated neoplasms [37]. Isoenzyme profiles were similar in malignant and nonmalignant tissues, although the enzyme isolated from ovarian cancers exhibited greater lability to heat and acid pH [37]. Interestingly, a decrease has been noted in serum a-1-fucosidase activity among ovarian cancer patients independent of stage, tumor burden, histologic grade or cell type [38]. Statistical analysis of enzyme levels in control and ovarian cancer populations suggests that the latter may have a 3-fold greater frequency of a genetic allele encoding low activity of serum 1-fucosidase. This observation may help to account for the earlier report that protein bound 1-fucose was increased in sera from ovarian cancer patients [39]. The concomitant expression of different carbohydrate determinants on mucin-like molecules may provide more specific markers than could be obtained by measuring any single determinant or enzyme activity. When several determinants have been characterized chemically, it may be possible to correlate the expression of different determinants with the activities of relevant enzymes. Ultimately, the abnormal expression of glycosyltransferases and glycosidases must be explained at the level of gene amplification, transcription or translation.

To date, a number of chromosomal abnormalities have been described in ovarian cancer cells [40–43], but relatively little is known regarding genetic abnormalities at a molecular level. In one recent report a mutant ras^K oncogene was found in a human epithelial ovarian carcinoma that was capable of transforming murine 3T3 cells [44]. Using the OC 125 monoclonal antibody, malignant and benign cells from the same ascites specimen could be distinguished. Only the malignant cells possessed transforming activity consistent with a somatic rather than a germ-line mutation. Both benign and malignant cells produced similar amounts of a p21 ras^K gene product. When the nucleotide sequence of the mutant ras^K gene has been obtained, it may be possible to prepare a monoclonal reagent that reacts with the aberrant polypeptide sequence in the mutant p21 protein which it specifies. Other investigators have examined expression of a variety of different onc-genes. In hybridization studies with fresh ovarian tumor RNA and ^{32}P labeled v-onc gene probes, 4 of 6 ovarian carcinomas expressed a ras^K gene [45].

Ras^K, myc and fos were expressed by all 6 tumors, whereas fems was expressed by 4 of 6 ovarian neoplasms. Expression of abl, fes, myb and src was not detected. Interestingly, more than one onc-gene was transcriptionally active in each of the tumors examined. In these cases, transformation may be associated with the increased production of multiple onc-gene products rather than the presence of a single mutant onc-gene. Whether or not onc-gene products will provide useful markers for epithelial ovarian cancers remains to be determined.

Markers defined by heteroantisera

In earlier studies with rabbit heteroantisera, radioimmunometric assays had been developed for two ovarian tumor associated glycoproteins OCA [46] and OCAA [47]. OCA appeared to cross-react with CEA, but a 70,000 dalton mioety (NB/70K) has been isolated from OCA preparations that is clearly distinct from CEA [48]. Two radioimmunometric assays for NB/70K have now been developed and evaluated clinically [49, 50]. With one assay, NB/70K levels were > 11 U/ml in 0 of 47 healthy controls, in 5% of 181 gynecology patients without known malignancy, in 88% of 8 patients with benign ovarian neoplasms, in 76% of 127 ovarian cancer patients following initial surgery and in 100% of 12 ovarian cancer patients where serum was obtained preoperatively [49]. The level of NB/70K correlated with increasing stage and residual tumor burden, but not with histology or grade. With an improved assay for NB/70K, > 10 U/50 ul serum was observed in 0 of 7 healthy controls, in 0 of 7 patients with benign ovarian tumors, in 4 of 23 patients with malignant nonovarian tumors and in 10 of 21 ovarian cancer patients [50]. Whether or not the marker correlates with clinical course has not yet been demonstrated. Development of monoclonal antibodies reactive with NB/70K (S. Knauf, personal communication) should facilitate further work with this marker.

Immunocytochemical detection of ovarian tumor cells in effusions

Two different reports suggest that monoclonal reagents might prove useful for detecting ovarian tumor cells in cytological preparations from ascites or pleural fluid [51, 52]. The Ca-1 antibody was originally developed from mice immunized with a lectin purified glycoprotein fraction from a laryngeal carcinoma cell line. The antigen consists of two components of 390,000 and 350,000 daltons [53]. The Ca antigen is expressed on malignant, but not benign somatic cell hybrids and can be detected in a wide variety of epithelial carcinomas including ovarian carcinoma as well as in some sarcomas

and some lymphomas. Ca is expressed on normal fallopian tube epithelium and transitional epithelium of the urinary tract. The monoclonal antibody Ca-1 correctly identified malignant cells in 21 of 25 cytological specimens which contained tumor cells [51]. Ovarian tumor cells were identified in each of 4 cases studied. Another monoclonal reagent, HMFG2 is directed against a determinant in the human milk fat globule, which is strongly expressed in the lactating mammary gland and in carcinomas of the breast, colon, lung and ovary [52]. AVA1 reacts with proliferating nonmalignant cells as well as adenocarcinomas of the colon, breast and ovary [52]. Cytological specimens were obtained from 27 patients with benign disease and from 38 cancer patients including 14 individuals with ovarian carcinoma. When conventional examination was compared to immunohistochemical stains, the latter confirmed the presence of malignant cells in 26 samples. In addition, immunohistochemical analysis confirmed the absence of malignant cells in 26 of 27 specimens from non-malignant effusions. In the remaining sample, immunohistochemistry suggested the presence of malignant cells which were found morphologically when the specimen was reexamined. Given the reactivity of the F36/22 antibody with breast carcinomas and virtually all ovarian tumors [26, 27], this reagent might also prove valuable for immunohistochemical analysis of effusions [27].

Radionuclide imaging with isotope-antibody conjugates

Earlier studies with heteroantisera suggested that radionuclide-antibody conjugates might prove valuable for localization of occult ovarian carcinomas. Imaging with ^{131}I-anti-CEA selected primary tumors in each of 13 patients [54]. Metastases could be identified in 6 of 9 cases whereas ultrasound or CT scan detected metastases in only 2 of these cases. More recently, ^{123}I-HMFG2 or ^{123}I-HMFG1 have been given to ovarian cancer patients prior to initial exploration [55]. Successful localization of large primary tumors has been achieved in each of 20 patients [56]. Given the uptake of radionuclide in the vascular pool and in liver, imaging of occult retroperitoneal and diaphragmatic metastases is likely to prove more difficult.

A variety of other reagents might be evaluated as carriers for radionuclides. Whether or not OC 125 or OC 133 will prove useful for imaging remains to be determined. Shed antigen might prevent antibody from reaching tumor sites. Studies in cell culture, however, suggest that ^{125}I-OC 125 remains firmly bound to the surface of 4 different epithelial ovarian carcinoma cell lines for up to 24 hrs without shedding or internalization [7]. Similar binding of OC 133 was observed with 3 of the same 4 cell lines. With the fourth line, however, $>70\%$ of OC 133 antibody was either shed or endocytosed during the same period. With all 4 lines specific antigens

recognized by OC 125 and OC 133 were found in spent culture medium, possibly released during cell death or by active secretion [7]. These data suggest that antigen may be lost from the cell surface by several different mechanisms and that antigen release is not inconsistent with binding of radiolabeled antibody to the tumor cell surface for prolonged periods. Such binding may be important for effective localization of radionuclides.

Conclusions

Development of the monoclonal technology has permitted the production of a variety of reagents which bind to antigenic determinants associated with epithelial ovarian carcinomas. None is specific for ovarian carcinoma, but several may prove valuable, particularly for monitoring response to treatment. The CA 125 assay may provide a useful marker for monitoring the course of epithelial ovarian cancer and deserves further evaluation in early detection of the disease. A number of monoclonal antibodies recognize carbohydrate determinants associated with high molecular weight mucin-like molecules. These determinants, in all probability, reflect the aberrant profile of goycosyltransferases and glycosidases within ovarian tumor cells. In recent studies, oncogene expression has been detected in epithelial ovarian cancers. Whether or not oncogene probes or products will prove useful in the management of ovarian carcinoma remains to be determined. Monoclonal reagents have been used to detect malignant cells in cytological specimens from effusions and have been used as carriers for radionuclides in imaging primary ovarian tumors and metastases. Use of monoclonal antibodies as carriers not only promises to provide more precise diagnosis but might also permit more effective treatment with conjugates containing drugs, toxins or large quantities of radioisotopes.

References

1. Bast RC Jr, Knapp RC: The immunobiology of ovarian carcinoma. In: Gynecologic Oncology, Griffiths CT, Fuller AF (eds). Boston: Martinus Nijhoff Publishers, 1983, pp 187–226.
2. Kohler G, Milstein C: Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256:495–497, 1975.
3. Bast RC Jr, Feeney M, Lazarus H, Nadler LM, Colvin RB, Knapp RC: Reactivity of a monoclonal antibody with human ovarian carcinoma. J Clin Invest 68:1331–1337, 1981.
4. Kabawat SE, Bast RC Jr, Bhan AK, Welch WR, Knapp RC, Colvin RB: Tissue distribution of a coelomic epithelium related antigen recognized by the monoclonal antibody OC 125. Int J Gyn Path 2:275–285, 1983.
5. Kabawat SE, Bast RC Jr, Welch WR, Knapp RC, Colvin RB: Immunopathologic characterization of a monoclonal antibody that recognizes common surface antigens of human

ovarian tumors of serous, endometrioid and clear cell types. Am J Clin Path 79:98–104, 1983.
6. Buick RN, Pullano R, Knapp RC, Urbach GI, Chang PL, Bast RC Jr: Classification of ovarian tumor associated antigens on the basis of distribution on cell populations undergoing differentiation. Proc Amer Assoc Cancer Res 25:40, (abstr), 1984.
7. Masuho Y, Zalutsky M, Knapp RC, Bast RC Jr: Interaction of monoclonal antibodies with cell surface antigens of human ovarian carcinomas. Cancer Res, 44:2813–2819, 1984.
8. Bast RC Jr, Klug TL, St. John E, Jenison E, Niloff J, Lazarus H, Berkowitz RS, Leavitt T, Griffiths CT, Parker L, Zurawski VR, Knapp RC: A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. N Engl J Med 309:883–887, 1983.
9. Klug TL, Bast RC Jr, Niloff JM, Knapp RC, Zurawski VR Jr: Monoclonal antibody immunoradiometric assay for an antigenic determinant (CA 125) associated with human epithelial ovarian carcinomas. Cancer Res 44:1048–1053, 1984.
10. Bast RC Jr, Klug TL, Schaetzl E, Lavin P, Niloff J, Greber TF, Zurawski VR Jr, Knapp RC: Monitoring human ovarian carcinoma with a combination of CA 125, CA 19-9 and carcinoembryonic antigen, Am J Obstet Gynecol, 149:553–559, 1984.
11. Chatal JF, Ricolleau G, Fumoleau P, Kramer M, Curtet C, Douillard JY: Radioimmunoassay of the CA 12-5 antigen in epithelial ovarian carcinomas. Cancer Detection Prevention 6:624, 1983.
12. Crombach G, Zippel HH, Wurz H: Clinical significance of cancer antigen 125 (CA 125) in ovarian cancer. Cancer Detection Prevention 6:623, 1983.
13. Kreienberg R, Melchart: CA 125-A new radioimmunoassay for monitoring patients with epithelial ovarian carcinoma. Cancer Detection Prevention 6:619, 1983.
14. Niloff JM, Klug TL, Schaetzl E, Zurawski VR Jr, Knapp RC, Bast RC JR: Elevation of serum CA 125 in carcinomas of the fallopian tube, endometrium, and endocervix. Am J Obstet Gynecol, 148:1057–1058, 1984.
15. Bast RC Jr, Siegal FP, Runowicz C, Klug TL, Zurawski VR Jr, Schonoholz D, Cohen CJ, Knapp RC: Elevation of serum CA 125 prior to diagnosis of an epithelial ovarian carcinoma. Gynecol Oncol, in press.
16. Niloff JM, Knapp RC, Schaetzl E, Reynolds C, Bast RC Jr: Ca 125 antigen levels in obstetric and gynecologic patients. Obstet Gynecol, in press.
17. Ruibal A, Encabo G, Miralles EM, Domenech FM: CA 125 seric levels in non ovarian pathologies. Med Clin (Barcelona) 82:500–501, 1984.
18. Bhattacharya M, Chatterjee SK, Barlow JJ, Fuji H: Monoclonal antibodies recognizing tumor-associated antigen of human ovarian mucinous cystadenocarcinomas. Cancer Res 42:1650–1654, 1982.
19. Colnaghi MI, Canaveri S, Dellatorre G, *et al.*: Monoclonal antibodies directed against human tumors. Proc 13th Int Cancer Congress P. 55, 1982.
20. Berkowitz RS, Kabawat S, Lazarus H, Colvin RC, Knapp RC, Bast RC Jr: Comparison of a rabbit heteroantiserum and a murine monoclonal antibody raised against a human epithelial ovarian carcinoma cell line. Am J Obstet Gynecol 146:607–612, 1983.
21. Mattes MJ, Cordon-Cardo C, Lewis JC Jr, *et al.*: Cell surface antigens of human ovarian and endometrial carcinoma defined by mouse monoclonal antibodies. Proc Natl Acad Sci (USA), in press.
22. Metzgar RS, Gaillard MT, Levske JJ, *et al.*: Antigens of human pancreatic adenocarcinoma cells defined by murine monoclonal antibodies. Cancer Res 42:601–608, 1982.
23. Metzgar RS, Rodriguez BS, Daasch VN, Fernsten PD, Meyers WC, Sindelar WF, Sandler RS, Seigler HF: Competition radioimmune assay for detection of a pancreatic cancer associated antigen (DU-PAN-2) in serum and ascited of patients with adenocarcinoma. Proc Natl Acad Sci (USA), in press.
24. Daasch VN, Fernsten PD, Metzgar RS: Radioimmunoassay studies of a monoclonal anti-

body to detect antigen (DUPAN-2) in the serum or ascites of adenocarcinoma patients. Proc Amer Assoc Cancer Res 23:266, 1982.
25. Papsidero LD, Croghan GA, O'Connell MJ, Valenzuela LA, Nemoto T, Chu TM: Monoclonal antibodies (F36/22 and M7/105) to human breast carcinoma. Cancer Res 43:1741–1747, 1983.
26. Croghan GA, Papsidero LD, Valenzuela LA, Nemoto T, Penetrante R, Chu TM: Tissue distribution of an epithelial and tumor-associated antigen recognized by monoclonal antibody F36/22. Cancer Res 43:4980–4988, 1983.
27. Croghan GA, Wingate MB, Gamarra M, Johnson E, Chu TM, Allen H, Valenzuela L, Tsukada Y, Papsidero D: Reactivity of monoclonal antibody F36/22 with human ovarian adenocarcinomas. Cancer Res 44:1954–1962, 1984.
28. Koprowski H, Steplewski Z, Mitchell K, Herlyn M, Herlyn D, Fuhrer JP: Colorectal carcinoma antigens detected by hybridoma antibodies. Somatic Cell Genet 5:957–972, 1979.
29. Magnani JL, Steplewski Z, Koprowski H, Ginsburg V: Identification of the gastrointestinal and pancreatic cancer-associated antigen detected by monoclonal antibody 19–9 in the sera of patients as a mucin. Cancer Res 43:5489–5492, 1983.
30. Del Villano BC, Brennan S, Brock P, Bucher C, Liu V, McClure M, Rake B, Space S, Westrick B, Schoemaker H, Zurawski VR Jr: Radioimmunometric assay for a monoclonal antibody-defined tumor marker, CA 19–9. Clinical Chem. 29/3:549–552, 1983.
31. Herlyn M, Sears HF, Steplewski Z, Koprowski H: Monoclonal antibody detection of a circulating tumor-associated antigen I. Presence of antigen in sera of patients with colorectal, gastric and pancreatic carcinoma. J Clin Immunol 2:135–140, 1982.
32. Charpin C, Bhan AK, Zurawski VR Jr, Scully RE: Carcinoembryonic antigen (CEA) and carbohydrate determinant 19–9 (CA 19–9) localization in 121 primary and metastatic ovarian tumors: An immunohistochemical study with the use of monoclonal antibodies. Int J Gynecol Path 1:231–245, 1982.
33. Bast RC Jr, Klug T, Knapp RC, Zurawski VR Jr: CA 125 and CA 19–9 can be expressed on the same mucin-like glycoprotein. Submitted for publication.
34. Chatterjee SK, Bhattacharya M, Barlow J: Glycosyltransferase and glycosidase activities in ovarian cancer patients. Cancer Res 39:1943–1951, 1979.
35. Waalkes TP, Rosenshein NB, Shaper JH, Ettinger DS, Woo KB, Paone JF, Gehrke CW: A feasibility study in the development of biological markers for ovarian cancer. J. Surgical Onc 21:207–214, 1982.
36. Gauduchon P, Tillier C, Guyonnet C, Heron JF, Bar-Guilloux E, Talaer JY: Clinical value of serum glycoprotein galactosyltransferase levels in different histological types of ovarian carcinoma. Cancer Res 43:4491–4496, 1983.
37. Chatterjee SK, Chowdhury K, Bhattacharya M, Barlow J: Beta-hexosaminidase activities and isoenzymes in normal human ovary and ovarian adenocarcinoma. Cancer 49:128–135, 1982.
38. Barlow JJ, DiCioccio RA, Dillard PH, Blumenson LE, Matta KL: Frequency of an allele for low activity of -L-Fucosidase in sera: Possible increase in epithelial ovarian cancer patients. JNCI 67:1005–1009, 1981.
39. Barlow JJ, Dillard PH: Serum protein-bound fucose in patients with gynecologic cancers. Obstet Gynecol 39:727–733, 1972.
40. Atkin NB, Baker MC, Robinson R, Gaze SE: Chromosome studies on 14 near-diploid carcinomas of the ovary. Eur J Cancer 10:143–146, 1972.
41. Kakati S, Hayata I, Oshimure M, Sandberg AA: Chromosomes and causation of human cancer and leukemia. X. banding patterns in cancerous effusions. Cancer 35:1729–1738, 1975.
42. Trent JM, Salmon SE: Karyotypic analysis of human ovarian carcinoma cells cloned in agar. Am J Human Genet 31:379 (abstr), 1979.
43. Whang-Peng J, Knutsen T, Douglass EC, Chu E, Ozols RF, Hogan WM, Young RC: Cyto-

genetic studies in ovarian cancer. Cancer Genetics and Cytogenetics 11:91–106, 1984.
44. Feig L, Bast RC Jr, Knapp RC, Cooper GM: Somatic activation of ras^k gene in a human ovarian carcinoma. Science 223:698–701, 1984.
45. Slamon DJ, deKernoin J, Verma IM, Cline M: Expression of cellular oncogenes in human malignancies. Science 224:256–252, 1984.
46. Knauf S, Urbach GI: A study of ovarian cancer patients using a radioimmunoassay for human ovarian tumor associated antigen OCA.Am J Obstet Gynecol 138:1222, 1223, 1980.
47. Bhattacharya M, Barlow JJ: Ovarian cystadenocarcinoma-associated antigen (OCAA). In: Compendium of Assays for Immunodiagnosis of Human Cancer. Herberman RB (ed). Amsterdam: Elsevier, North Holland Inc, pp 527–431, 1979.
48. Knauf S, Urbach GI: Identification, purification and radioimmunoassay of NB/70K, a human ovarian tumor-associated antigen. Cancer Res 41:1351–1357, 1981.
49. Chang P-L, Urbach GI, Dembo AJ: Circulating levels of ovarian cancer antigen NB/70K: Clinical correlations. Proc Amer Soc Clin Oncol 3:2, 1984.
50. Knauf S, Taillon-Miller P, Helmkamp F, Bonfiglio TA, Beecham JB: Selectivity for ovarian cancer of an improved serum radioimmunoassay for human ovarian tumor-associated antigen NB/70K. Gynecologic Oncol 17:349–355, 1984.
51. Woods JC, Sprigas AI, Harris H, McGee JO'D: A new marker for human cancer cells. 3. Immunocytochemical detection of malignant cells in serous fluids with the Ca-1 antibody. Lancet 2:512–514, 1982.
52. Epenetos AA, Canti G, Taylor-Papadimitriou J, Curling M, Bodmer WF: Use of two epithelium-specific monoclonal antibodies for diagnosis of malignancy in serous effusions. Lancet 2:1004–1006, 1982.
53. Bramwell ME, Bhavanadant VP, Wiseman G, Harris H: Structure and function of the Ca antigen. Br J Cancer 48:177–183, 1983.
54. Van Nagell JR Jr, Kim E, Casper S, *et al.*: Radioimmunodetection of primary and metastatic ovarian cancer using radiolabeled antibodies to carcinoembryonic antigen. Cancer Res 40:502–506, 1980.
55. Epenetos AA, Britton KE, Mather S, Shepherd J, Granowska M, Taylor-Papadimitriou J, Nimmon CC, Durbin H, Hawkins LR, Malpas JS: Targeting of iodine-123 labelled tumour-associated monoclonal antibodies to ovarian, breast, and gastrointestinal tumours. Lancet 2:999–1004, 1982.
56. Shephard JH, Epenetos AA, Britton KE, *et al.*: Radioimmune diagnosis of ovarian carcinoma using tumor associated monoclonal antibodies. Proc Soc Gyn Oncol 14:12, 1983.

3. The human tumor clonogenic assay used to study ovarian cancers

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1. Introduction

During the last decade, the treatment of ovarian cancer has changed markedly as the available armamentarium of cytotoxic drugs has improved. It remains apparent that surgical cytoreduction (debulking) procedures are useful, particularly in those cases where the largest mass of residual tumor can be reduced to less than 1 cm in size [1]. However, even more important than the degree of surgical debulking which can be achieved is the apparent improvement in clinical response rates seen with new innovations in chemotherapy. The Gynecologic Oncology Group (GOG) conducted a large randomized trial between 1976 and 1979 of suboptimal (>3 cm tumor bulk) untreated ovarian cancers (Protocol # 22), which showed that melphalan alone was as effective as the combination of cyclophosphamide and doxorubicin in the observed response rates and survival durations [2]. However, the subsequent GOG Protocol # 47 showed that the addition of cisplatin to cyclophosphamide and doxorubicin improved response rates from 46% to 71% with the median duration of survival increasing from 9.5 months to 15.0 months [3]. The fact, however, becomes apparent that a survival duration of 15 months is not a cure, and the good benefits which cisplatin bestowed on patients with ovarian cancers are limited. The empiric choice of chemotherapeutic agents does not necessarily provide maximal benefit to each patient with ovarian cancer.

Therefore, great enthusiasm was generated by a novel application of laboratory technology to clinical oncology with the advent of the Human Tumor Clonogenic Assay (HTCA). Since the description by Hamburger and Salmon in 1977 of a technique to grow human solid tumors *in vitro* [4], the HTCA has been used to test cells from ovarian cancer patients with cytotoxic agents, looking for correlations between laboratory predictive data and patient responses to therapy [5, 6]. Fortunately, there are often several drugs which do show cytotoxic activity against ovarian tumor cells, both *in vitro*

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and *in vivo*, as is necessary if this new laboratory tool is to be useful in planning patient therapy.

In this chapter we will look at three areas related to HTCA studies of ovarian cancers: (1) Recently published data describing *in vitro* results from several laboratories that have grown human ovarian cancers in the HTCA will be presented. For clinicians considering the potential usefulness of the HTCA in ovarian cancer therapy, it is helpful to know what percentage of patient tumors tested in the HTCA can be expected to grow and thereby yield drug sensitivity information. (2) Application of the *in vitro* drug data to clinical oncology will be considered, looking particularly at prospectively determined correlations between the HTCA and patient treatment responses. Although the degree of drug sensitivity or resistance determined *in vitro* is important, there are additional factors which impinge on patient responses to therapy, such as grade of tumor, tumor bulk, and patient performance status. Any truly useful predictive tool for determining probable responses to therapy must include more information about the patient than simply the drug sensitivity numbers. (3) New investigational studies which are now possible using HTCA technology will be presented. These include areas of multiple drug interactions in combination therapy, innovative methods of drug administration, and the screening of Phase II and preclinical compounds for cytotoxic activity.

2. Growth of human ovarian cancers in the HTCA

Great efforts have been made by many cell biologists to establish human ovarian carcinoma cells as permanent cell lines *in vitro*. In spite of many investigators laboring for several decades toward this goal, there are less than a score of established ovarian cancer cell lines reported in the literature [7–13]. The rarity of such cell lines precludes the conclusion that any of them are truly representative of the broad spectrum of patient tumors. It is therefore of particular significance that the HTCA now permits *in vitro* study of ovarian cancers.

The methods of the HTCA have been partially standardized by many laboratories, and have been reported in detail in the literature [6, 14]. A few essential features of the assay must be emphasized. Viable tumor cells must be obtained from the patient and transported to the laboratory as quickly as possible. There is decreasing viability of cells in a tumor nodule when there is prolonged transit time from the operating room to the laboratory, particularly apparent when overnight transport of tumor specimens is necessary. Cells must be disaggregated into a single cell suspension, using either mechanical or enzymatic means [15]. The cell suspension is then exposed to cytotoxic agents in varying concentrations, either for one hour incubation

prior to plating in agar or by incorporating the drug into the agar at the time of plating, achieving continuous drug exposure. After plating in agar, the cells are incubated at 37 °C for 10 to 21 days, until colonies are seen in the culture dish (defined as aggregates of > 50 cells). Histologic verification that the cell colonies growing in agar actually represent tumor cells is obtained by fixing and staining the culture plates after the colonies have been counted [16].

Colony growth is compared among culture dishes which have been treated with drugs and with the untreated control cultures. A decrease in colony growth in drug treated cultures compared to control cultures is attributed to cytotoxic effects of drugs. Empirically defined endpoints are of drug sensitivity when colony survival is reduced to less than 30% of control levels, and of drug resistance when colony survival is greater than 50% of the control level. Between 30% and 50% colony survival is an intermediate level of sensitivity [17].

An experiment using a patient's tumor for drug studies is considered to be successful when greater than 30 colonies can be counted in the control cultures, and enough cells have been available for testing at least one drug.

Results published from several laboratories growing ovarian cancers in the HTCA are summarized in Table 1. It is of note that there is a significant latitude of successes among the laboratories. Although methods of the HTCA are becoming standardized, there still are problems providing optimal *in vitro* growth conditions for ovarian cancer cells *in vitro*. One of the *in vivo* cell growth regulating mechanisms is the interaction of cell subpopulations [24]. Experiments demonstrating this phenomenon *in vitro* have included the depletion or addition of macrophages to cultures, which does alter tumor cell growth [6]. Adding a feeder layer of macrophages incorpo-

Table 1. Successful growth in the HCTA of human ovarian cancers

Author	Institution	No. specimens attempted	No. % grown	Successful growth
Williams [18]	Mayo Clinic	138	60	43%
Sikic [19]	Stanford University	82	52	64%
Natale [20]	University of Michigan	52	34	65%
Von Hoff [21]	University of Texas	110	79	72%
Hamburger [6]	University of Arizona	31	25	84%
Ozols [22]	National Cancer Institute	40	34	85%
Welander [23]	Bowman Gray School of Medicine	149	139	93%

Growth is defined as > 30 colonies per well in control plates with enough cells to test at least 1 drug.

rated beneath the agar underlayer has been shown to improve tumor cell colony growth in ovarian cancers up to 180% [25].

Another technical modification of the HTCA has been separation of cells prior to plating, using a Ficoll-Paque (R) (Pharmacia, Piscataway, New Jersey) gradient. This facilitates removal of non-viable cells and cellular debris resulting from the tumor disaggregation procedure [26]. There are probably deleterious effects on the overall culture growth when a large number of dead cells are present in the agar. If these are removed prior to plating, an overall enhancement of cell growth is observed in most cultures.

Using both the feeder layer and the gradient method of cell separation prior to plating, the Gynecologic Oncology Laboratory at Bowman Gray School of Medicine has been able to grow 93.3% of ovarian cancer specimens sent to the laboratory [23]. This is a higher percentage of successful growth than many laboratories have achieved, and suggests that careful attention to technical details when preparing tumor cells for culture seems to be beneficial. Clearly, a majority of ovarian cancer specimens can be grown in the assay and can be studied for patterns of drug sensitivity.

3. Correlations of HTCA data and patient treatment responses

3.1. Low in vitro response rates to standard chemotherapeutic agents

In the evaluation of a new laboratory tool, one of the first questions to answer is whether the *in vitro* information correlates with historical clinical experience. In order to address this point, drugs commonly used in clinical trials have been tested in the HTCA. Clinical treatment protocols have demonstrated that our present 'first line' drugs in ovarian cancer therapy include cisplatin and doxorubicin [3]. When these drugs are tested in the HTCA with tumor cells from patients having ovarian cancers, surprisingly low levels of activity are seen (Table 2). In contrast, some 'second line' drugs have been tested and show slightly higher degrees of cytotoxic activity *in vitro* (Table 2). When we relate traditionally accepted clinical response rates to new HTCA data, there are often wide variations in the common clinically used doses and schedules of administration, which will thereby alter the ultimate response rates. Two conclusions can be drawn from a review of HTCA data, one being that the degree of effectiveness of our present drugs is very low. The other conclusion is that the HTCA can be used to screen a wide variety of agents, to determine which ones, in general, are the most active in ovarian cancers, and which are specifically the most active for an individual patient who requires treatment.

Table 2. Standard drugs for ovarian cancer therapy tested in the human tumor clonogenic assay

'First line' drugs	No. assays	No. sensitive & intermediate (%)	No. resistant (%)
Doxorubicin	70	11 (15.7%)	59 (84.3%)
Cisplatin	71	12 (16.9%)	59 (83.1%)

'Second line' drugs	No. of assays	No. sensitive & intermediate (%)	No. resistant (%)
5-Fluorouracil	99	46 (46.5%)	53 (53.5%)
Vinblastine	69	20 (29.0)	49 (71.0%)
Vincristine	90	28 (31.1%)	62 (68.9%)
Mitomycin-C	61	21 (34.4%)	40 (65.6%)

All assays have been done using cells from patients who have not had these particular drugs before.

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3.2. Single agent drug testing in the HTCA and the observed clinical correlations

Beginning in the late 1970s with some of the first HTCA studies, retrospective correlations have been made between the laboratory and patient responses. By definition, a clinical response in a patient requires that a measurable tumor shrink in size by at least 50% for greater than one month duration. Stable tumor size or a shrinkage less than 50% does not constitute a valid response. The *in vitro* definition of a response using HTCA data requires tumor cell colony reduction to less than 30% of the control level. The intermediate response category is colony survival between 31% and 50%, with the resistant category being colony survival greater than 51% of control levels. Results of the earliest published series from the University of Arizona report 95 correlations from single agent chemotherapy trials, showing a majority of resistant responses after primary chemotherapy [27]. Combining the Arizona report with other published series, the total group has shown 98% correlation for the prediction of resistance and 75% for the prediction of clinical response in previously treated patients (Table 3). These series include only a small number of patients, due to the fact that

Table 3. Correlations between human tumor clonogenic assay results and patient responses to single agent chemotherapy in ovarian cancers

Author	No. trials	S/S (*in vitro/in vivo*)	S/R	R/S	R/R
Alberts [27]	95	14	7	1	73
Natale [20]	28	10	3	1	14
Welander [23]	19	9	1	0	9
Total	142	33	11	2	96

Predictive accuracy: Sensitive -75%; Resistant -98%.

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most chemotherapy for ovarian cancer is now given as combinations of drugs rather than as single agents. It seems clear that the correlative accuracy of the HTCA with patient responses is high enough to be clinically useful, when single agents are being considered for patient treatment. It is also clearly demonstrated that our choices of drugs have low levels of activity. Prediction of drug resistance can, therefore, be done with great accuracy.

3.3. Changes in patient survival when HTCA data is used to plan therapy

In the previous section it is noted that patient responses to therapy can be predicted with a certain degree of accuracy. A more significant issue is whether these responses result in improved patient survival. From the University of Arizona, Alberts has reported the results of chemotherapy based upon HTCA data in 37 patients with ovarian cancers who were relapsing from prior therapy [28]. All patients' tumors were tested in the HTCA. Twenty-two had at least one active drug identified in the assay and received that drug as treatment. The combined complete responders and partial responders totalled 73% with a 12.6 month median survival duration. The remaining 15 patients in this series had no effective drugs identified by the HTCA and were, therefore, treated with empirically selected drugs. Among these patients the complete responders and partial responders totalled 20% with a 4.5 month median survival duration.

3.4. In vitro and in vivo correlations when multiple drugs are used in combination

As the biology of tumor cell growth and responses to therapy becomes

better understood, it is clear that tumors have heterogeneous subpopulations of cells with different growth rates and degrees of drug sensitivity [29]. The empiric clinical means to deal with that problem is to administer multiple drugs to patients, either simultaneously or in an alternating fashion, in an attempt to avoid the selection of a resistant subpopulation of tumor cells. Response rates of advanced ovarian cancers rose from 37.5% when single agent melphalan was used [27] to 71% with the combination of cisplatin, doxorubicin and cyclophosphamide [3].

The HTCA was initially used to test single drugs, correlated with patient treatment regimens using only one drug. Now, a further clinical application of the HTCA requires that it be used to plan combination chemotherapy for patients.

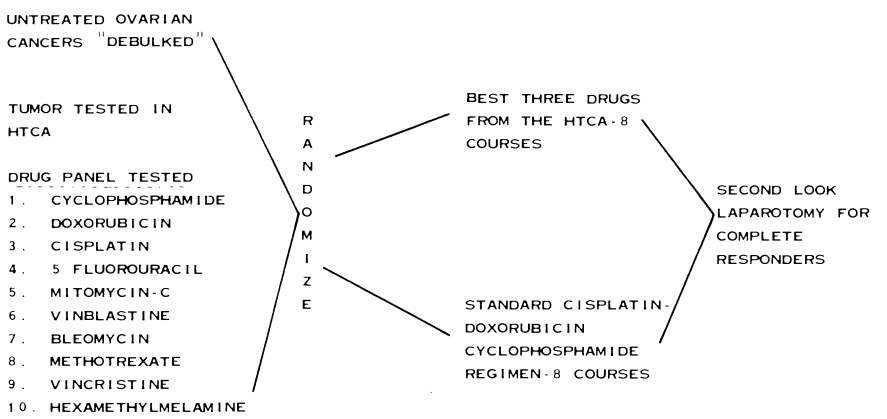
When several drugs are combined *in vitro* in the HTCA, there are interactions of the combination that are not always predictable. One published report studied combinations of two drugs *in vitro*, using cells from 15 ovarian, 5 uterine cancers and 1 testicular cancer [30]. In that series combined cisplatin/vinblastine and combined doxorubicin/cisplatin showed added effects in 53% and 67% of the *in vitro* trials, respectively. Another report studied three drug combinations, including cisplatin, doxorubicin, cyclophosphamide tested with ovarian cancer cells. The overall colony reduction of this drug combination was similar to that seen when the best agent in this combination was tested alone [31]. These studies seem to suggest that clear drug synergy *in vitro* may be difficult to demonstrate.

One clinical trial is currently being done by the Piedmont Oncology Association (POA # 83182), determining in a prospective fashion whether the HTCA can select active drugs as effectively as the empirically determined cisplatin/doxorubicin/cyclophosphamide regimen. In summary the protocol requires that tumor cells from advanced untreated ovarian cancer patients be tested in the HTCA with a panel of 10 cytotoxic agents, each tested singly. According to the percent colony survival seen with each of the 10 drugs in the HTCA, a protocol combining the 'best three drugs' from the HTCA will be compared to the standard cisplatin/doxorubicin/cyclophosphamide regimen in randomized patients (Table 4). This study will investigate whether drugs showing single agent activity *in vitro* can be combined into a successful clinical treatment protocol. This study will add significant information to help determine the ultimate clinical usefulness of the HTCA as applied to the currently popular multiple drug treatment regimens.

3.5. Predictive clinical features correlated with responses to therapy

Any new predictive assay needs to be correlated with clinical responses if it is going to be useful in a clinical setting. Very often many host factors

Table 4. Protocol schema for prospectively selecting the 'best drugs' from the HTCA vs. standard cisplatin-doxorubicin-cyclophosphamide therapy for advanced untreated ovarian carcinomas



Patients are stratified prior to randomization, depending upon HTCA results; those with *no* drugs tested having <50% colony survival are 'resistant'. Those patients having drugs with <50% colony survival are 'sensitive'.

Data will be analyzed at the completion of the study to consider 1) bulk of residual tumor following debulking and 2) performance status.

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impinge upon patient responses to therapy, in addition to the degree of tumor cell drug sensitivity predicted by the HTCA. To ignore all of the host factors as we evaluate a new *in vitro* predictive test can strongly bias the conclusions that we reach.

Response rates in ovarian cancer therapy have been related to at least three clinical parameters. 1) Tumor grade has been shown to correlate highly with response rates. Patients having well differentiated tumors are more likely to respond to chemotherapy than those with poorly differentiated tumors [32, 33]. 2) In advanced ovarian cancers, there is an inverse correlation between the size of the largest remaining tumor nodules following cytoreductive surgery and survival [1]. The GOG has defined optimal disease as those cases in which the largest residual tumor mass is less than 1 cm. Those other cases which have residual tumor nodules greater than that 1 cm are classified as suboptimal. 3) Patient performance status is also highly correlated with responses to chemotherapy [34]. Those ovarian cancer patients who are unable to remain out of bed usually do not tolerate chemotherapy well enough for responses to be seen, even in cases where there is a degree of *in vitro* sensitivity reported.

A review of the clinical features shown by 48 ovarian cancer patients who were seen at Bowman Gray School of Medicine using the 'best drugs' determined by the HTCA is shown in Table 5. The three parameters discussed are shown, each correlated independently with clinical response. When an individual patient has more than one poor prognostic indicator, the chance for a favorable treatment response is remote, even though there may be drugs which appear active in the HTCA. Statistical work is now being done to correlate these variables into a formula for predicting responses, which will incorporate the degree of drug sensitivity from the HTCA with these other clinically observed host factors.

3.6. Biologic variations in tumor responses to therapy

It is not known whether there are inherent biologic differences among tumors, if we compare those which show a degree of sensitivity to certain drugs in the HTCA with those which are totally resistant to all drugs tested.

Table 5. Predictive clinical features correlated with responses in 48 patients treated with the 'best drugs' selected by the HTCA

Tumor grade (FIGO classification)		
Grade	CR + PR (%)	Stable + progression
I	4 (67)	2
II	5 (83)	1
III	17 (57)	13
Unknown	2 (33)	4
Performances status (GOG classification)		
Perf. status	CR + PR (%)	Stable + progression
0	2 (67)	1
1	12 (71)	5
2	13 (72)	5
3	1 (10)	9
Residual tumor bulk (following cytoreductive surgery)		
Residual tumor	CR + PR (%)	Stable + progression
<1 cm (optimal)	7 (100)	0
>1 cm (suboptimal)	21 (51)	20

Volm *et al.* from Germany have published the work of a collaborative group looking to see whether *in vitro* tumor response to an index drug will predict the same response to any other drug given to that patient [35]. Tumors from 72 patients having ovarian cancers were studied in this series, by incubating for three hours *in vitro* a single cell suspension of tumor cells with either doxorubicin or cyclophosphamide. The degree of cell death induced by doxorubicin *in vitro* was correlated with the patient's clinical response to a regimen of cyclophosphamide and 5-fluorouracil as follows: the test showed sensitivity to doxorubicin and the patient responded to cyclophosphamide/5-fluorouracil 53% of the time; the test showed resistance to doxorubicin and the patient failed to respond to cyclophosphamide/5-fluorouracil 96% of the time. Median patient survival was also different in that those patients showing *in vitro* sensitivity to doxorubicin had a median survival duration of 318 days, compared to 183 days in those patients showing *in vitro* resistance. These authors suggest that this *in vitro* assay is determining the proliferation rate of the tumor cell population, which will significantly affect tumor response to *any* cytotoxic agent. In contrast with the HTCA, this assay is not intended to determine the most effective cytotoxic agent, but only to determine the probability that any cytotoxic drug is likely to be effective.

4. New investigational studies using HTCA technology

4.1. Studies of interferon cytoxicity in the HTCA

Since ovarian carcinoma cells have been demonstrated to grow well in the HTCA, they can be used as a source of human tumor material for investigational studies of new drugs. Within the broad category of biologic response modifiers is human leukocyte interferon, which has had many observed effects on cell growth. In high concentrations it appears to have cytotoxic effects on some human tumor cells [36]. The application of recombinant DNA technology to the production of purified human leukocyte interferon has now made available adequate quantities of recombinant human interferon alpha$_2$ (rIFNα_2) for both *in vitro* and *in vivo* testing. Of 38 ovarian carcinomas tested with rIFNα_2 (Schering Corporation) by the Gynecologic Oncology Laboratory at Bowman Gray School of Medicine, 12 patients (31.6%) have shown colony reduction to levels below 50% of the control cultures. Another report using rIFNαA (Hoffmann-La Roche) and ovarian carcinoma cells showed 32.7% of the tumors tested exhibiting less than 50% colony survival [37].

Of greater interest is the observation that combinations of interferon and various cytotoxic drugs, used either sequentially or concurrently show

increased cytotoxicity in an additive and occasionally synergistic fashion [38]. Various combinations and schedules of agents have been used. One method has been pretreatment of cells with rIFNα_2 followed by exposure to doxorubicin. Of six ovarian carcinomas studied there have been three which showed either synergistic or additive effects of the sequential regimen [39]. Two cells lines of ovarian carcinomas have also been tested in sequential and combined continuous drug exposure. Synergy has been demonstrated in both cell lines using the combinations of both doxorubicin/rIFNα_2 and cisplatin/rIFNα_2 [39]. The mechanism of interferon action in such examples remains unclear. There does appear to be some schedule dependence in these combinations which will maximize the cytotoxic effect. Continued efforts to explore such drug interactions are being done, attempting to define the scope of activity of these combinations and the optimal concentrations and schedule of administration.

4.2. Intraperitoneal administration of chemotherapy

It is axiomatic to state that greater concentrations of cytotoxic drugs will induce greater tumor cell kill. Clinical experience would suggest that 'more is better' in terms of drug concentrations achievable in patients. The HTCA is able to give information about concentration related degrees of cell survival following drug exposure. One application of this information can be to plan innovative techniques of drug administration, such as intraperitoneal (IP) chemotherapy for ovarian cancer patients. Since ovarian cancer spreads by surface implantation throughout the peritoneal cavity, it is possible to use a treatment method exposing tumor cells directly to cytotoxic drugs. The concentration of drugs within the peritoneal cavity can be increased up to 1,000 fold above the maximum safe achievable plasma level [40–42]. This concept of IP chemotherapy is related to the HTCA in that increasing degrees of tumor cell sensitivity to higher drug concentrations can be identified and predicted in advance. Conversely, there are some tumor cells which remain resistant to drugs in the HTCA at any concentration which might reasonably be given. It is helpful, when feasible, to obtain HTCA drug sensitivity information prior to selection of a drug for IP administration, to determine whether there is any probability of a favorable tumor response using high concentrations of the drug.

4.3. Phase II drug studies using the HTCA

The ongoing effort of chemotherapy research is to improve the effectiveness of available drugs. Clinical trials of new experimental drugs are done by

first testing a new drug in Phase I studies, which will determine the maximum tolerable dose levels and the associated toxicity problems. Phase II trials are then carried out, using the determined maximum safe dose to treat a variety of tumor types. Present ethical practices, in general, prevent the administration of 'experimental' drugs to any patient except those who have failed 'standard' therapy. It is probable that drugs which might have some degree of activity in patient therapy could be missed when clinical testing is restricted to patients who are in poor overall condition from relapsing cancers.

In contrast, the HTCA can be used to screen new drugs, using tumor cells from any patient, whether pretreated or not [43]. The cost and time-saving afforded by *in vitro* testing can be very significant. We have shown in Section 3.2 of this chapter that the correlation of HTCA resistance with clinical nonresponse is very high. Therefore, the failure of a drug to show activity in the HTCA can spare many patients unnecessary toxicity from Phase I and II testing. When activity is demonstrated in the HTCA, then further patient testing is necessary to determine the drug doses and scheduling which will maximize patient responses.

Ovarian cancers are an area of active investigation of Phase II drugs. Clinical studies of some of these agents have been done by the GOG. A GOG publication has reported etoposide (VP-16-213) treatment results showing two of 24 patients with ovarian cancers (8.3%) who had a partial response [44]. Other GOG studies still in progress are evaluating aziridinylbenzoquinone (AZQ) and mitoxantrone. Comparative HTCA results of these and several other Phase II drugs tested with ovarian cancer cells are shown in Table 6. It seems clear that most of the available new drugs do not

Table 6. Phase II agents tested in the HTCA with ovarian carcinoma cells

Drug	Method of drug exposure	No. patients	Sensitive (%)	Intermediate (%)	Resistant (%)
AZQ	Continuous	15	2 (13.3%)	1 (6.7%)	12 (80.0%)
Mitoxantrone	1 hour	34	7 (20.5%)	3 (8.8%)	24 (70.5%)
Mitoxantrone	Continuous	22	0 (0.0%)	3 (13.6%)	19 (86.4%)
Pentamethylmelamine	1 hour	16	2 (12.5%)	2 (12.5%)	12 (75.0%)
Pentamethylmelamine	Continuous	40	0 (0.0%)	4 (10.0%)	36 (90.0%)
VP-16	Continous	16	1 (6.3%)	0 (0.0%)	15 (93.7%)
Spirogermanium	1 hour	12	0 (0.0%)	0 (0.0%)	12 (100.0%)
Bisantrene	Continous	16	0 (0.0%)	0 (0.0%)	16 (100.0%)

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have great cytotoxic activity for ovarian cancers. Continued screening will be necessary in order to find other more active compounds.

4.4. Preclinical drug testing using the HTCA

Perhaps the greatest long term impact of the HTCA on clinical oncology will be in the area of new compound screening for cytotoxic activity. We are acutely aware of the low level of activity of our presently available group of drugs for patient therapy. Therefore, any assistance in more quickly identifying new active compounds will impact widely on patient care.

In 1980 the Division of Cancer Treatment, National Cancer Institute, began a study in four laboratories, screening new compounds using the HTCA technique with human tumor cells. One of the types of tumors studied has been ovarian cancer. All compounds are provided to the participating laboratories as 'blinded' specimens. Of the first 158 compounds tested, all of which were thought to be inactive by prior animal tumor screening, these laboratories correctly identified 153 (96.8%) as inactive in the HTCA. There are five compounds which were thought to be inactive in the preliminary animal tumor screening, but did show significant activity in the HTCA. These will now go on to further screening to determine a more accurate spectrum of activity [45]. It is anticipated that the HTCA can become an economical and efficient part of the new drug evaluation process.

5. Conclusion

Reviewing published studies using the HTCA to test human ovarian cancers, 60 to 80% of the tumor specimens sent to the laboratory should grow and be able to be tested for drug sensitivity. While this expected growth rate is not as high as is desired, it does permit an optimistic expectation for chemotherapists and patients alike that information can be obtained for most patients whose tumors are studied in the HTCA. Sufficient numbers of correlations between HTCA data and patient responses have been made using single agent therapy in order to establish credibility of the assay in patients with previously untreated ovarian cancer. The usefulness of the HTCA applied to multiple drug combinations is still being studied in randomized trials.

As important as clinical trials are to patient therapy, a greater future impact of the HTCA on clinical oncology will be in investigative studies. The screening of compounds for cytotoxic activity, the study of drug interactions, and the determination of optimal drug concentrations and schedul-

ing will probably benefit more patients than the present efforts aimed at individualizing a patient's therapy.

Problem areas that remain fall into two categories. One concerns optimal *in vitro* conditions for growth of tumor cells. The logistics of getting tumor material to the laboratory for HTCA studies become complicated from outlying hospitals. There is not yet any good transport medium available which will keep cells alive well enough to permit overnight shipping from distant communities. Even specimens which are promptly delivered to the laboratory will not grow in all cases. Work continues in this area, attempting to improve the technical capability preparing specimens and growing them in agar.

The other problem area concerns the relative ineffectiveness of our present chemotherapy drugs. When most of the agents available for clinical use show responses in less than 20% of cases, it is often difficult to identify active drugs in patient screening. Our clinical goal of long-term control of tumor growth remains elusive when our drugs are so limited in effectiveness. Combination chemotherapy is an effort to get around this problem, but still needs to be studied further in the HTCA in order to maximize responses.

In spite of these problem areas of HTCA techniques, there is a solid core of evidence already established telling us that the HTCA can usefully be applied to the identification of effective drugs for treating patients with ovarian cancer. Additional work will broaden our understanding of drug effects and, hopefully, will identify new and more potent anti-tumor agents for clinical use.

References

1. Griffiths CT: Surgical resection of tumor bulk in the primary treatment of ovarian carcinoma. National Cancer Institute Monograph 42:101–104, 1975.
2. Omura GA, Blessing JA, Morrow CP, Buchsbaum HJ, Homesley HD: Follow-up on a randomized trial of melphalan vs. melphalan plus hexamethylmelamine vs. adriamycin plus cyclophosphamide in advanced ovarian adenocarcinoma. Proc Am Soc Clin Oncol 22:470, 1981.
3. Omura GA, Ehrlich CE, Blessing JA: A randomized trial of cyclophosphamide plus adriamycin with or without cisplatinum in ovarian carcinoma. Proc Am Soc Clin Oncol 1:104, 1982.
4. Hamburger AW, Salmon SE: Primary bioassay of human tumor stem cells. Science 197:461–463, 1977.
5. Salmon SE, Hamburger AW, Soehnlen B, Durie BGM, Alberts DS, Moon TE: Quantitation of differential sensitivity of human tumor stem cells to anticancer drugs. N Engl J Med 298:1321–1327, 1978.
6. Hamburger AW, Salmon SE, Kim MB, *et al.*: Direct cloning of human ovarian carcinoma cells in agar. Cancer Res 38:3438–3444, 1978.

7. Fogh J, Trempe G: New Human tumor cell lines. In: Human tumor cells in vitro, Fogh J (ed), New York, Plenum Press, 1975, pp 115–159.
8. DiSaia PJ, Morrow M, Kanabus J, Piechal W, Townsend DE: Two new tissue culture lines from ovarian cancer. Gynecol Oncol 3:215–219, 1975.
9. Sinna GA, Beckman G, Lundgren E, Nordenson I, Roos G: Characterization of two new human ovarian carcinoma cell lines. Gynecol Oncol 7:267–280, 1979.
10. Kimoto T, Ueki A, Nishitani K: A human malignant cell line established from ascites of patient with embryonal carcinoma of ovarium. Acta Pathol Jpn 25:89–98, 1975.
11. Freedman RS, Pihl E, Kusyk C, Gallager HS, Rutledge F: Characterization of an ovarian carcinoma cell line. Cancer 42:2352–2359, 1978.
12. Yamada T: The cellular biology of a newly established cell line of human ovarian adenocarcinoma in vitro. Keio J Med 23:53–70, 1974.
13. Woods LK, Morgan RT, Quinn LA, Moore GE: Comparison of four new cell lines from patients with adenocarcinoma of the ovary. Cancer Res 39:4449–4459, 1979.
14. Soehnlen B, Young L, Liu R: Standard laboratory procedures for in vitro assay of human tumor stem cells. In: Cloning of human tumor stem cells, Salmon SE (ed), New York, Alan R. Liss, Inc., 1980, pp 331–338.
15. Pavelic ZP, Slocum HK, Rostum YM, Creaven PJ, Karakousis C, Takita H: Colony growth in soft agar of human melanoma, sarcoma, and lung carcinoma cells disaggregated by mechanical and enzymatic methods. Cancer Res 40:2160–2164, 1980.
16. Salmon SE, Buick RN: Preparation of permanent slides of intact soft-agar colony cultures of hematopoietic and tumor stem cells. Cancer Res 39:1133–1136, 1979.
17. Moon TE: Quantitative and statistical analysis of the association between in vitro and in vivo studies. In: Cloning of human tumor stem cells, Salmon SE (ed), New York, Alan R. Liss, Inc., 1980, pp 209–221.
18. Williams TJ, Lieber MM, Podratz KC, Malkasian GD Jr: Soft agar colony formation assay for in vitro testing of sensitivity to chemotherapy of gynecologic malignancies. Am J Obstet Gynecol 145:940–947, 1983.
19. Sikic BI, Evans TL, MacKintosh FR, Smith HS: Human tumor clonogenic assays – studies of growth and drug sensitivity. Proc Amer Assoc Cancer Res 23:186, 1982.
20. Natale RB, Kushner B: Applications of the human tumor cloning assay to ovarian cancer. Proc Amer Assoc Cancer Res 22:156, 1981.
21. Von Hoff DD, Cowan J, Harris G, Reisdorf G: Human tumor cloning: feasibility and clinical correlations. Cancer Chemother Pharmacol 6:265–271, 1981.
22. Ozols RF, Willson JKV, Grotzinger KR, Young RC: Cloning of human ovarian cancer cells in soft agar from malignant effusions and peritoneal washings. Cancer Res 40:2743–2747, 1980.
23. Welander CE, Homesley HD, Jobson VW: *In vitro* chemotherapy testing of gynecologic tumors: Basic for planning therapy? Am J Obstet Gynecol 147:188–195, 1983.
24. Buick RN, Fry SE, Salmon SE: Effect of host cell interactions on clonogenic carcinoma cells in human malignant effusions. Br J Cancer 41:695–704, 1980.
25. Welander CE, Natale RB, Lewis JL Jr: *In vitro* growth stimulation of human ovarian cancer cells by xenogeneic peritoneal macrophages. J Natl Cancer Inst 69:1039–1047, 1982.
26. Gaines JT, Welander CE, Homesley HD: Improved cloning efficiency in the human tumor stem cell assay using Ficoll gradient separation. Proc Amer Assoc Cancer Res 24:311, 1983.
27. Alberts DS, Chen HSG, Soehnlen B, Salmon S, Surwit EA, Young L: *In vitro* clonogenic assay for predicting response of ovarian cancer to chemotherapy. Lancet (Aug 16) 340–342, 1980.
28. Alberts DS, Chen HSG, Young L, *et al.*: Improved survival for relapsing ovarian cancer patients using the human tumor stem cell assay to select chemotherapy. Proc Amer Soc Clin Oncol 22:462, 1981.

29. Coldman AJ, Goldie JH: A mathematical model of drug resistance in neoplasms. In: Drug and hormone resistance in neoplasia, Vol. 1, Bruchovsky N, Goldie JH (eds), Boca Raton, Florida, CRC Press, Inc., 1982, pp 55–78.
30. Alberts DS, Salmon SE, Surwit EA, Chen HSG, Moon TE, Meyskens FL: Combination chemotherapy *in vitro* with the human tumor stem cell assay. Proc Amer assoc Cancer Res 22:153, 1981.
31. Sondak VK, Korn El, Morton DL, Kern DH: Absence od *in vitro* synergy for chemotherapeutic combinations tested in the clonogenic assay. Proc Amer Assoc Cancer Res 24:316, 1983.
32. Day TG, Gallager HS, Rutledge FN: Epithelial carcinoma of the ovary: prognostic importance of histologic grade. National Cancer Institute monograph 42:15–18, 1975.
33. Ozols RF, Garvin AJ, Costa J, Simon RM, Young RC: Histologic grade in advanced ovarian cancer. Cancer Treatment Reports 63:255–263, 1979.
34. Stanley KE: Prognostic factors for survival in patients with inoperable lung cancer. J Natl Cancer Inst 65:25–32, 1980.
35. Volm M: *In vitro* short-term test to determine the resistance of human tumors to chemotherapy. Cancer 48:2127–2135, 1981.
36. Epstein LB, Shen JT, Abele JS, Reese CC: Sensitivity of human ovarian carcinoma cells to interferon and other antitumor agents as assessed by an *in vitro* semisolid agar technique. Ann NY Acad Sci 350:228–244, 1980.
37. Salmon SE, Durie BGM, Young L, Liu RM, Trown PW, Stebbing N: Effects of cloned human leukocyte interferons in the human tumor stem cell assay. J Clin Oncol 1:217–225, 1983.
38. Aapro MS, Alberts DS, Salmon SE: Interaction of human leukocyte interferon with vinca alkaloids and other chemotherapeutic agents against human tumors in clonogenic assay. Cancer Chemother Pharmacol, 10:161–166, 1983.
39. Welander C, Gaines J, Homesley H, Rudnick S: *In vitro* synergistic effects of recombinant human interferon alpha$_2$ and doxorubicin on human tumor cell lines. Proc Amer Soc Clin Oncol 2:42, 1983.
40. Dedrick RL, Myers CE, Bungay PM, DeVita VT Jr: Pharmacokinetic rationale for peritoneal drug administration in the treatment of ovarian cancer. Cancer Treatment Reports 62:1–11, 1978.
41. Jones RB, Myers CE, Guarino AM, Dedrick RL, Hubbard SM, DeVita VT: High volume intraperitoneal chemotherapy (2 belly bath') for ovarian cancer. Cancer Chemother Pharmacol 1:161–166, 1978.
42. Howell SB, Pfeifle CL, Wung WE, *et al.*: Intraperitoneal cisplatin with systemic thiosulfate protecion. Ann Int Med 97:845–851, 1982.
43. Salmon S, Alberts D, Meyskens F: A new concept. *In Vitro* phase II trial with the human tumor stem cell assay. Proc Am Soc Clin Oncol 21:329, 1980.
44. Slayton R, Petty W, Blessing J: Phase II trial of VP-16 in treatment of advanced ovarian adenocarcinoma. Proc Amer Assoc Cancer Res 20:190, 1979.
45. Shoemaker Rh, Wolpert-Defilippes MK, Makuch RW, Venditti JM: Use of the human tumor clonogenic assay for new drug screening. Proc Amer assoc Cancer Res 24:311, 1983.

4. Cytoreductive surgery for ovarian cancer

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Ovarian cancer presents a unique surgical challenge because of its mode of dissemination. Early in its course, epithelial ovarian cancer is usually asymptomatic, and even small primary tumors may exfoliate cells which implant throughout the peritoneal cavity. At the time of diagnosis, approximately 70–80% of epithelial tumors have spread beyond the ovaries. Germ cell tumors may also disseminate in this manner, but because they are more rapidly growing, they are frequently symptomatic, so are more commonly diagnosed early.

For tumors spread beyond the ovaries, initial surgical resection usually involves cytoreduction rather than complete surgical excision. Cytoreductive or debulking surgery is a procedure whereby a surgically incurable tumor is partially removed to improve the effectiveness of subsequent therapy, usually chemotherapy or radiation.

Apart from carcinoma of the ovary, cytoreductive surgery has been advocated for carcinoma of the testis, Burkitt's lymphoma, sarcoma, renal cell carcinoma, tumors of the adrenal gland and other endocrine related tumors, and neoplasms of the central nervous system. However the available evidence suggests that only in carcinoma of the ovary and Burkitt's lymphoma is there any significant benefit [1].

Rational for cytoreductive surgery

Because metastatic ovarian cancer cannot be cured surgically, subsequent chemotherapy or radiation therapy are mandatory. Skipper and colleagues determined that for an exponentially growing tumor, the killing effect of chemotherapeutic agents was a logarithmic function, that is, a given dose killed a constant fraction, not a constant number, of cells, regardless of the cell population initially present [2]. Theoretically, therefore, the greater the initial cell population, the more cylces of chemotherapy should be required

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to eradicate residual disease, but with a sufficient number of cycles, cure should be obtained.

The reason that ovarian and other metastatic malignant neoplasms are not usually cured by chemotherapy relates to the fact that they develop resistance to the chemotherapeutic agents. The question is whether this resistance, which may be either temporary or permanent, can be modified by cytoreductive surgery.

Temporary resistance may be related to either physiologic barriers or alterations in the cellular kinetics [3]. For solid tumors, adequate drug diffusion to all cells is fundamental for cytotoxicity. In animal studies, Gullino and Grantham reported a 15-fold reduction in the blood supply of hepatomas compared to normal liver [4]. They stated that if the blood flow of hepatomas in rats and mice follows the same laws which regulate the general circulation, it could be expected that a substance injected into the host would circulate roughly 20 times through the liver before passing once through the hepatoma. This poor tumor circulation is reflected in the very low content of free glucose and high levels of lactic acid in the interstitial fluid of solid tumors [5]. As tumors grow, the blood supply to the central region becomes particularly tenuous, ultimately manifesting as tumor necrosis. However, there are adjacent viable, though poorly perfused areas, and these may act as pharmacologic sanctuaries.

The ability of chemotherapeutic agents to penetrate solid ovarian tumor masses has not been evaluated, but some recent experimental evidence suggests that the limited ability of methotrexate to penetrate solid tumor masses, with resulting limited tissue concentrations of the drug, offers an alternative explanation for the limited effectiveness of methotrexate when used as an adjuvant for osteosarcoma [6]. Optimal cytoreduction, particularly if all gross tumor is removed, should significantly reduce treatment failure due to the presence of pharmacologic sanctuaries.

The second cause of temporary resistance to chemotherapy relates to the alterations in cellular kinetics associated with large tumor masses. As large tumors outstrip their blood supply, there is a decrease in the growth fraction of the tumor [7].

The growth fraction is the proportion of proliferating cells in a population, and it can be estimated by an autoradiographic method following the parenteral injection of tritiated thymidine [8]. The reason a high growth fraction is important is that nonproliferating cells (G_0) are not susceptible to cell-cycle specific chemotherapeutic agents, while alkylating agents and DNA binders (such as Cis-platinum) react with or bind to DNA regardless of cell cycle phase during exposure, but apparently kill only those neoplastic or normal cells that attempt DNA replication prior to repair [9]. Thus, for all chemotherapeutic agents, a high growth fraction is necessary to ensure optimal cell kill, and this phenomenon is also exploitable by cytoreductive surgery.

Permanent resistance to chemotherapy is due mainly to spontaneous mutation to phenotypic drug resistance, and occurs as an intrinsic property of genetically unstable malignant cells. Goldie and Coldman developed a mathematical model, which related the drug sensitivity of tumors to their spontaneous mutation rate [10]. The development of a resistant clone by the tumor is a random event, dependent on the growth curve of the tumor and the mutation rate. As tumor size increases, the probability of resistant clones increases, so that the expectation of cure will depend on the size the tumor has reached from the initial transformation to the time when therapeutic intervention begins.

Although nothing can be done to overcome the permanent resistance due to spontaneous genetic mutation, epigenetic phenomena may also induce permanat drug resistance. Exposure to chemotherapeutic agents per se may produce drug resistance. A tumor nodule with a volume of 1 cc will contain about 1 billion cells [11]. The larger the tumor volume prior to initiation of therapy, the greater the number of cycles that will be needed to eradicate the disease thus increasing the likelihood of induced resistance. In addition, a lengthy period of chemotherapy may accelerate the growth of a resistant population by enhancing its mutation rate [3]. Initial optimal cytoreduction, followed by intensive combination chemotherapy, should help to overcome both of these problems.

The final theoretical advantage of aggressive cytoreductive surgery is that it may enhance the immunocompetence of the patient. Morton regarded surgery as a form of immunotherapy, pointing out that in many patients, the major role of the surgeon is to remove the bulk of the tumor in order to lower the level of immunosuppression induced by the neoplasm [12]. Cell-mediated immunity [13] and blocking factor activity [14] have both been shown to have a direct relationship to tumor burden in female genital tract malignancies.

Preoperative management

A presumptive diagnosis of ovarian cancer can be made in any patient who has a pelvic mass, together with an abdominal mass or ascites. Such patients should have a complete blood count, serum electrolytes, serum creatinine, blood urea nitrogen, liver function tests, clotting studies, chest x-ray, intravenous pyelogram, barium enema, and electrocardiogram. An upper gastrointestinal study need not be done routinely, but should be performed if there are symptoms referable to the stomach, as about 5% of ovarian malignancies are metastatic from another primary. Similarly, mammograms should be obtained if there are any suspicious breast lumps, as ovarian metastases may occur even with a small primary breast cancer.

Pelvic and abdominal CT scans or ultrasonic examinations are not helpful, as such patients require exploratory laparotomy regardless of the results of such investigations. Similarly, liver, bone, or brain scans are not helpful unless there are specific symptoms or signs to suggest involvement of these organs. A Papanicolaou smear should be obtained to exclude cervix cancer.

When laboratory results are available, appropriate measures should be taken to correct abnormal values. Blood transfusion is indicated if the hematocrit is less than 30%, and acid-base or electrolyte imbalance should be corrected appropriately. In patients who are clinically malnourished, which will usually imply weight loss of greater than 10% of their body weight, total parenteral nutrition should be commenced about 10 days preoperatively. This will restore a positive nitrogen balance, enhance wound healing, and significantly decrease postoperative morbidity. The subclavian route is preferred for central catheter placement. Using hypertonic (25%) dextrose and aminoacids, together with daily intralipid infusions, between 3200 and 3500 Cal can be given over a 24 hour period. Hyperalimentation should be used with caution in patients with diabetes mellitus, and those with impaired renal or hepatic function.

As the surgical incision may extend from the symphysis pubis to the epigastrium, shallow breathing and reluctance to cough may be expected postoperatively, thus significantly increasing the likelihood of pulmonary complications. All patients with a history of chronic chest disease, and all heavy smokers, should have arterial blood gas measurements preoperatively, to establish a baseline for comparison with postoperative values. In addition, smoking should be discontinued at least one week preoperatively, and intensive chest physical therapy given.

Table 1. Bowel preparation

Preoperative Day No. 3
Low residue diet
Colace, 1 capsule at 6 p.m.
Preoperative Day No. 2
Clear liquid diet
Magnesium citrate 100 cc at 8 a.m.
Tap water enema at night $\times 2$
Preoperative Day No. 1
Clear liquid diet
Magnesium citrate 100 cc at 8 a.m.
P.O. Neomycin 1 gm q4h $\times 4$ doses
P.O. Erythromycin base 1 gm q4h $\times 4$ doses
Tap water enemas until no solid stool at night
Commence I.V. fluids at 8 a.m. to correct fluid and electrolyte imbalance caused by bowel cleansing.

A thorough mechanical and antibiotic bowel preparation should be performed on all patients, because bowel resection may be required as part of the cytoreduction. Our bowel preparation is shown in Tabel 1.

Broad spectrum prophylactic antibiotics are used, eg. cephoxitin 1 gram, 2 hours preoperatively and 1 gram every 8 hours for 3 doses postoperatively. Prophylactic mini-dose heparin is used. A Swan-Ganz catheter is inserted to ensure accurate fluid and electrolyte balance, particularly if the patient has significant ascites or a history of heart disease. Four units of packed cells should be typed and crossmatched.

Surgical objectives

The surgical objective when operating on patients with advanced ovarian cancer is to perform total (or subtotal) abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy, appendectomy, and removal of as many metastatic tumor nodules as possible. Ideally, one would like to render the patient free of all gross disease, but if this is not feasible, an attempt should be made to reduce all individual tumor nodules to less than 1.5 cm in diameter (i.e. 'optimal' cytoreduction). In order to achieve optimal cytoreduction, it may be necessary to perform bowel or urologic procedures, such as partial bowel resection, or resection of distal ureter and transureteroureterostomy. It is important that the surgical team be prepared to undertake such operations.

The quantity of disease removed at the time of surgery is much less important than the amount of disease left behind. Significant benefits accrue to the patient only if the residual individual tumor nodules are less than about 1.5 cm in diameter [15–18]. Therefore, if on exploration of the abdomen, it is not considered feasible to achieve optimal cytoreduction, the surgical goals should still be to remove the primary disease and omentum, in order to improve patient comfort, but bowel surgery should be performed only to relieve obstruction, and urologic procedures would not be indicated. Removal of a large omental cake in patients with ascites will frequently prevent or decrease the accumulation of ascitic fluid postoperatively.

With judicious use of the end-to-end stapling device, it is almost invariably possible to clear the pelvis of bulk disease, and even the largest of omental cakes can be resected safely unless it is invading the splenic hilum or diaphragm. Therefore, inability to achieve optimal cytoreduction will usually result from large disease in the upper abdomen, particularly disease involving the diaphragm, liver, porta hepatitis, or lesser omentum. Occasionally, a matted mass of retroperitoneal lymph nodes invading the underlying vessels will be unresectable.

Surgical techniques

The operation should be performed under general anesthesia with the patient usually in the supine position. If, on pelvic and rectal examination under anethesia, there is fixed disease in the cul-de-sac, suggesting the possible need for rectosigmoid resection, the operation should be performed with the patient in the low lithotomy (or 'ski') position, to facilitate subsequent bowel reanastomosis with the EEA stapler.

A midline or paramedian incision, extending from the symphysis pubis to the epigastrium is used. There is no place for attempting cytoreductive surgery through a transverse lower abdominal incision. Upon entering the peritoneal cavity thorough exploration should be performed, and the surgical goals elucidated, in line with the previously stated principles. The remainder of the description will assume that optimal cytoreduction is considered feasible.

Regarding the pelvic dissection, it may be possible to perform total abdominal hysterectomy and bilateral salpingo-oophorectomy in the standard manner, or with minor modifications only. However, frequently the tumor mass is densely adherent to pelvic organs, and cannot be separated from the pelvic peritoneum, thereby grossly distorting the anatomy. There may also be loops of bowel adherent to the tumor mass, which must be initially dissected free, to allow the bowel to be packed out of the pelvis.

When the pelvic anatomy is grossly distorted, extirpation of the pelvic tumor is facilitated by a retroperitoneal approach [19]. The dissection is started by incising the posterior parietal peritoneum just lateral to the external iliac vessels, and opening the retroperitoneal space bilaterally. Each ureter is identified as it crosses the bifurcation of the common iliac artery, and kept under direct vision throughout the pelvic dissection. Each infundibulopelvic ligament is isolated and ligated early in the dissection, to help decrease blood loss.

Removal of the pelvic tumor is facilitated by continuing the peritoneal incisions anteriorly until they meet over the bladder, distal to the tumor mass. Posteriorly the peritoneal incisions meet anterior to the rectum and sacrum, beyond the tumor mass. Tumor bearing peritoneum can usually be dissected off the bladder or rectal muscle. The ureters are dissected to the level of the uterine arteries, the latter are ligated, and then total or subtotal hysterectomy can be performed in continuity with the tumor mass. We prefer to perform a supracervical hysterectomy in the presence of extensive pelvic disease, to avoid the possibility of subsequent tumor growth at the apex of the vagina.

If the tumor is growing into the rectum, sigmoid colon, or sigmoid mesentery, low anterior resection of the colon in continuity with the pelvic mass will be required. It is desirable to transect the sigmoid colon early in the

dissection with the GIA (gastrointestinal anastomosis) stapler, then ligate and divide the vessels in the sigmoid mesentery, in order to gain access to the presacral space. With blunt dissection in the presacral space, it is possible to get below the pelvic tumor, which can then be elevated after transecting the rectal pillars between the presacral and pararectal spaces. This allows access to the distal rectum. As much distal rectum as possible should be spared when the bowel is transected below the tumor. Primary bowel reanastomosis should be performed using the EEA stapler, and a protecting colostomy is required only if the patient has received previous pelvic radiation [20].

Occasionally, the pelvic tumor will be invading the lower urinary tract. If the bladder is invaded, partial cystectomy with primary repair is required. If the distal ureter is involved, it should be resected in continuity with the mass, and reconstruction of the lower urinary tract performed. If only the distal two or three centimeters are resected, ureteroneocystostomy may be performed, the bladder being suspended from the pelvic sidewall by a psoas hitch. If a larger segment of ureter is resected, transureteroureterostomy is required [21].

At the end of the pelvic dissection, any enlarged pelvic lymph nodes are removed, then an attempt is made to reperitonealize the pelvis. At times there may be no available peritoneum, and sigmoid colon and cecum may be utilized to help cover the denuded pelvis, and prevent loops of small bowel from becoming adherent. If no coverage can be obtained, reepithelialization will eventually occur spontaneously.

Attention is then turned to the upper abdomen. If there is no gross involvement of the omentum, infracolic omentectomy may be satisfactory, but if an omental cake is present, total omentectomy should be performed. Although an omental cake may appear to be invading the transverse colon, it can usually be dissected free to expose the lesser sac and transverse mesocolon. The omentum is then taken off the greater curvature of the stomach, care being taken to ligate all short gastric vessels. Traction downwards and medially on the omental cake facilitates its removal from the region of the stomach and gastrosplenic ligament. At times the tumor will invade very close to the hilar region of the spleen, necessitating careful dissection around the splenic pedicle, and rarely splenectomy.

After removing the pelvic tumor and omentum, any grossly involved para-aortic lymph nodes should be excised, together with nodules greater than 1.5 cm in diameter on the bowel serosa or parietal peritoneum. Ovarian cancer usually 'cakes' onto serosal surfaces rather than invading deeply, so lines of cleavage can usually be found. However, if there are large tumor masses involving the small bowel mesentery, or invading beyond the serosa, partial small bowel resection should be performed. In order to save time, during an otherwise long operation, stapling devices may be used to expe-

dite the reanastomosis [20]. Because prolonged ileus is a common complication of extensive cytoreductive surgery, placement of a gastrostomy tube will significantly improve the patient's postoperative comfort by obviating the need for a nasogastric tube.

Metastases to the diaphragm are usually diffuse and not resectable. However, on two occasions we have encountered an isolated diaphragmatic nodule two or three centimeters in diameter, removal of which would leave the patient with virtually no gross disease. On both occasions, we have mobilized the liver by transecting the triangular ligament, and resected the nodule. A chest tube is placed and attached to an underwater seal drain, and the defect in the diaphragm is repaired with interrupted 0 black silk sutures. Both patients treated in this manner recovered uneventfully.

Following tumor resection, peritoneal irrigation with normal saline is carried out, and the wound closed with Smead-Jones sutures. Drains are avoided if possible, but if considered desirable, we prefer 10 mm Jackson-Pratt drains. It is important that the surgeon carefully records the site and size of all residual tumor nodules at the end of the operation.

Postoperative care

Many of these patients are very ill and require intensive care nursing postoperatively. All patients need careful monitoring of their fluid and electrolyte balance and urine output, particularly during the first 72 hours. Urine output per catheter should be measured hourly, and maintained at greater than 30 ml per hour.

Incentive spirometry should be commenced on the day of surgery, and be supervised every 4 hours during waking hours. An occasional patient with chronic lung disease will require intubation and ventilation for the first 24 to 48 hours postoperatively.

Total parenteral nutrition should begin on the first postoperative day provided the patient is afebrile. Prophylactic minidose heparin is continued until the patient is fully mobile, but antibiotics are discontinued after 24 hours, unless a specific infection is being treated. Early ambulation is encouraged, but oral fluids are withheld until flatus is passed. Early oral intake will only prolong the postoperative ileus. If a colonic resection has been performed, suppositories and enemas are contraindicated.

Discussion

Although cytoreductive surgery is a rational initial approach to the management of advanced ovarian cancer, it has remained controversial because

no prospective study has yet been reported to prove its value. Critics contend that patients whose disease is most favourable initially are the ones who can be optimally cytoreduced, and that they would have had a more favourable prognosis regardless of the cytoreduction. We have recently written a protocol for a prospective study of cytoreductive surgery, to be carried out by the multi-institutional Gynecologic Oncology Group, but it will be several years before the results of this study are available.

In the absence of prospective data, several retrospective studies have shown that patients with small residual disease (individual tumor nodules less than 1.5 cm in diameter) following primary surgery have longer median survivals than patients with larger residual disease [15–18, 22–24] (Table 2). Among patients with small residual disease (i.e. those having optimal cytoreduction), survival is further improved if disease can be reduced to 0.5 cm or less (Figure 1).

Table 2. Survival versus amount of residual disease following cytoreductive surgery

			Median survival (mos.)	
Author	Number	% Optimal	Optimal	Suboptimal
Griffiths et al. [15]	102	72%	28.6	11
Wharton et al. [23]*	104	43%	27.6	15.3
Hacker et al. [22]	47	66%	22	6
Stehman et al. [25]*	56	30%	39	22.5

* Not all patients operated on at author's institution.

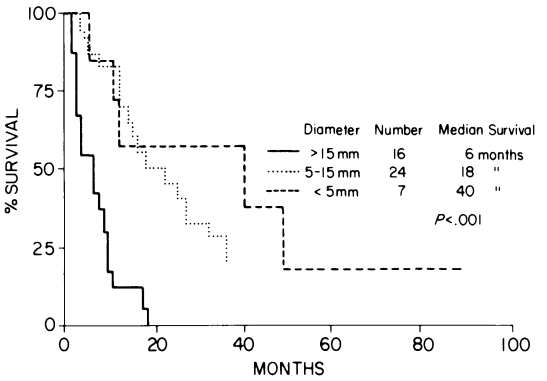

Figure 1. Survival versus diameter of largest residual disease. Broken line = diameter > 1.5 cm, 16 patients with median survival six months, dotted line = diameter 0.5–1.5 cm, 24 patients with median survival 18 months, solid line = diameter <0.5 cm, seven patients with median survival 40 months. $P<.001$. Source: Hacker et al. [22]. Reproduced with permission of the American College of Ob-Gyn.

Although overall response to chemotherapy is not dependent on the amount of residual disease, complete responses are uncommon unless residual disease is small. This therapeutic advantage for patients with small residual disease seems to be particularly true for patients receiving combination chemotherapy (Tables 3, 4). Patients who achieve a complete response to primary chemotherapy are the only ones likely to be cured of their disease.

Apart from the diameter of the largest residual disease, other factors significantly affecting survival, in our experience, are the presence or absence of ascites, and the diameter of the largest metastatic disease prior to cytoreductive surgery. Figure 2 shows that within the optimal group, patients without ascites had a median survival of 32 months, compared to 12 months for those with ascites. Similarly, within the optimal group, median

Table 3. Response to single agent chemotherapy versus amount of residual disease

		Complete response	
Author	Chemotherapy	Optimal	Suboptimal
Wharton et al. [23]	Melphelan	12/45 (27%)	8/59 (14%)
Wharton et al. [26]	Hexamethylmelamine	5/17 (29%)	3/37 (8%)
Young et al. [27]	Melphelan	2/11 (18%)	4/26 (15%)
Hacker et al. [22]	Melphelan	5/31 (16%)	0/16 (0%)
Schwartz and Smith [28]	Melphelan	16/51 (31%)	15/90 (17%)
Total		40/155 (26%)	30/228 (13%)

Table 4. Response to multi-agent chemotherapy versus amount of residual disease

		Complete response	
Author	Chemotherapy	Optimal	Suboptimal
Griffiths et al. [16]	Adriamycin cyclophosphamide	9/12 (75%)	0/3 (0%)
Young et al. [27]	Hexacaf*	8/8 (100%)	5/32 (16%)
Greco et al. [29]	H-CAP or H-FAP†	14/16 (86%)	3/29 (10%)
Ehrlich et al. [30]	PAC [2]	6/13 (46%)	7/22 (32%)
Edwards et al. [31]	HAC [3] or Melphalan + P [4]	31/67 (46%)	16/86 (19%)
Total		68/116 (58.6%)	31/172 (18%)

* Hexamethylmelamine, Cyclophosphamide, Methotrexate, 5-Flourouracil.

† Hexamethylmelamine, Adriamycin, Cisplatin, and Cyclophosphamide or 5-Flourouracil.

[2] Cisplatin, Adriamycin, Cytoxan.

[3] Hexamethylmelamine, Adriamycin, Cyclophosphamide.

[4] Cisplatin.

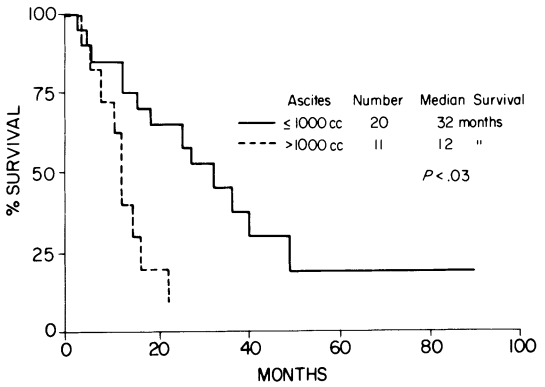

Figure 2. Survival of patients in optimal group versus presence of clinical ascites. Solid line = ascites ≤1000 ml, 20 patients with median survival 32 months; broken line = ascites > 1000 ml, 11 patients with median survival 12 months. $P<.03$. Source: Hacker et al. [22]. Reproduced with permission of the American College of Ob-Gyn.

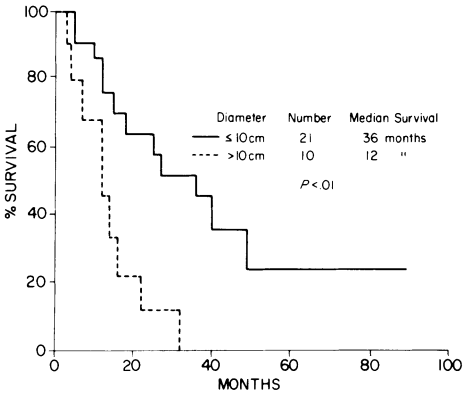

Figure 3. Survival of patients in optimal group versus diameter of largest metastatic disease before cytoreduction. Solid line = diameter ≤10 cm, 21 patients with median survival 36 months, broken line = diameter > 10 cm, ten patients with median survival 12 months. $P<.01$. Source: Hacker et al. [22]. Reproduced with permission of the American College of Ob-Gyn.

survival was 36 months if largest metastatic disease was 10 cm or less prior to cytoreduction, compared to 12 months if disease was greater than 10 cm (Figure 3). No patient with metastatic disease greater than 10 cm in diameter was cured of disease. This observation is consistent with the Goldie-Coldman hypothesis that in bulky tumors, spontaneous mutation will have already resulted in clones of cells permanently resistant to chemotherapeutic agents, thereby eliminating the possibility of cure, regardless of the extent of the surgical resection [10]. Our results are based on experience with single agent (alkeran) chemotherapy, and it may be expected that results would improve with a non cross-resistant combination of drugs. Although cure

may not be possible for such patient, meaningful prolongation of life may be achieved with aggressive primary surgery and subsequent chemotherapy.

Proper cytoreductive surgery for ovarian cancer requires an operator thoroughly conversant with the relevant gynecologic, gastrointestinal, and urologic procedures, and knowledgeable about the management of the disease. Such patients should be referred to oncology centers to ensure an adequate operation. When performed by an experienced operator, about four-fifths of patients with advanced ovarian cancer can have their tumor optimally cytoreduced.

One criticism of cytoreductive surgery has been that it is associated with unacceptable morbidity. In our experience, we have had no operative mortality, and most of the morbidity has been associated with pulmonary infection, fluid and electrolyte imbalance, bowel dysfunction, and delayed wound healing. Bowel and urologic procedures have not increased morbidity [21, 32]. With more liberal use of Swan-Ganz catheters to monitor fluid balance, and hyperalimentation to improve nutrition, morbidity should decrease further in the future.

Quality of life is an important consideration when dealing with a disease with a low likelihood of cure. Blyth and Wahl [33] studied the quality of life in patients having cytoreductive surgery for advanced ovarian cancer, and reported good quality of life in 15 of 19 patients (79%) whose disease was optimally cytoreduced, compared to 7 of 15 patients (46.6%) who had larger residual disease following their primary surgery. They concluded that aggressive cytoreductive surgery actually improved the quality of life for patients with advanced ovarian cancer.

Secondary cytoreductive surgery

Because most patients will not have a complete response to first line chemotherapy, an important question in the management of the incomplete or nonresponders is whether there is any value in a secondary attempt at cytoreductive surgery, to be followed by second line therapy, such as second-line chemotherapy or whole abdominal radiation.

Phillips and colleagues reported secondary laparotomy with attempted cytoreduction on 26 patients who had shown a partial response to primary therapy [34]. Subsequent survival was clearly related to the amount of residual disease following the secondary operation. Patients who had residual disease two centimeters or less in diameter had a mean survival of 44 months, compared to about 6 months for those with larger residual nodules. In eleven of the 26 patients, radiation alone had been used as primary therapy. Therapy following the secondary operation was individualized.

They concluded that the morbidity and mortality of a properly conceived and performed secondary laparotomy were low, and that the benefits far outweighed the risks.

Schwartz and Smith also reported that resection of residual tumor at second-look operation influenced subsequent survival [28]. Thirty-nine patients who had total removal of residual tumor at the second-look operation had 2-year and 5-year survival rates 47.5% and 27.0%, respectively, while 36 patients who had partial tumor resection with residual nodules less than 2 cm in diameter had 2-year and 5-year survival rates of 29.5% for each interval. If residual tumor following second-look operation was greater than 2 cm in diameter, the 2-year and 5-year survival rates were 9.0% for each interval.

Raju and colleagues reported on 65 patients with epithelial ovarian tumors who had an incomplete initial operation, but had shown at least a partial clinical response to cis-platinum containing chemotherapy [35]. At the end of the primary surgery, 52 of their patients still had the uterus in situ and 36 still retained one or both ovaries. At the end of the secondary laparotomy, an additional 36 hysterectomies were performed and one or both ovaries were removed in 27 patients. Of 38 patients who had a partial response to first-line chemotherapy, as determined at the secondary laparotomy, 9 patients had all macroscopic disease removed at the secondary laparotomy, but survival expectancy for these 9 patients was no better than that of the other 29 partial responders in whom residual tumor was left. They concluded that secondary cytoreductive surgery was not beneficial, and may have actually shortened survival for patients who had no regression on initial chemotherapy. One reason for their findings may be that most of their patients received cis-platinum containing combination chemotherapy initially, and were continued on the same therapy following the surgery. Their findings suggest that change of therapy is necessary if any benefits are to accrue from aggressive secondary cytoreduction.

In a review of our UCLA experience, Berek and colleagues reported on 32 patients who underwent secondary cytoreductive surgery for advanced stage epithelial ovarian cancer [36]. Optimal cytoreduction was possible in 12 patients (38%) and necessitated bowel resection in 17 (53%). There was no operative mortality. Median survival for patients in the optimal group was 20 months which was significantly longer than the 5 months median survival for the suboptimal group ($P < .01$). The surgery was particularly beneficial in patients who had no clinical evidence of ascites, and small tumor masses (less than 5 cm in diameter) following completion of primary therapy. One of the reasons for the apparent benefit of secondary cytoreduction in this group may be that almost half of the patients received single alkylating agents only as primary therapy, so that potentially effective second-line therapies were available to them.

Summary

Although contravening traditional surgical principles, and suffering from a lack of prospective data, there is both theoretical and statistical justification for advocating aggressive primary cytoreductive surgery for patients with advanced stage ovarian cancer. In fact, given the limited ability of chemotherapy or radiation to cure bulky ovarian cancer, it is likely that the availability of proper primary surgical effort for such patients may be the single most important determinant of their ultimate prognosis. The available data on secondary cytoreductive surgery is very limited, but would suggest that patients with relatively small residual tumor masses following first line chemotherapy may benefit from their surgical removal, particularly if there is a subsequent change in therapy.

References

1. Silberman AW: Surgical debulking of tumors. Surg Gynecol Obstet 155:577, 1982.
2. Skipper HE, Schabel FM Jr, Wilcox WS: Experimental evaluation of potential anticancer agents XII: On the criteria and kinetics associated with 'curability' or experimental leukemia. Cancer Chemother Rep 35:1–111, 1964.
3. De Vita VT: The relationship between tumor mass and resistance to chemotherapy. Implications for surgical adjuvant treatment of cancer. Cancer 51:1209, 1983.
4. Gullino PM, Grantham FH: Studies on the exchange of fluids between host and tumor. II. The blood flow of hepatomas and other tumors in rats and mice. J Natl Cancer Inst 27:1465–1491, 1961.
5. Gullino PM, Clark SH, Grantham FH: The interstitial fluid of solid tumors. Cancer Research 24:780–794, 1964.
6. West GW, Weichselbaum R, Little JB: Limited penetration of methotrexate into human osteosarcoma spheroids as a proposed model for solid tumor resistance to adjuvant chemotherapy. Cancer Research 40:3665, 1980.
7. Tannock I: Cell kinetics and chemotherapy. A critical review. Cancer Treat Rep 62(8):1117, 1978.
8. Mendelsohn ML: Autoradiographic analysis of cell proliferation in spontaneous breast cancer of C3H mouse. III. The growth fraction. J Natl Cancer Inst 28:1015, 1962.
9. Skipper HE: Thoughts on cancer chemotherapy and combination modality therapy (1974). JAMA 230(7):1033, 1974.
10. Goldie JH, Coldman AJ: A mathematic model for relating the drug sensitivity of tumors to their spontaneous mutation rate. Cancer Treat Rep 63:1727, 1979.
11. De Vita VT: Cell kinetics and the chemotherapy of cancer. Cancer Chemother Rep 3(2):23, 1971.
12. Morton DL: Changing concepts in cancer surgery; surgery as immunotherapy. Am J Surg 135:367, 1978.
13. Khoo SK, Tillack SV, Mackay EV: Cell-mediated immunity: Effects of female genital tract cancer, pregnancy, and immunosuppressive drugs. Aust NZ J Obstet Gynaecol 15:156, 1975.
14. Pattillo RA, Ruckert ACF, Story MT, *et al.*: Immunodiagnosis in ovarian cancer: Blocking factor activity. Am J Obstet Gynecol 133:791, 1979.

15. Griffiths CT: Surgical resection of tumor bulk in the primary treatment of ovarian carcinoma. Natl Cancer Inst Mong 42:101, 1975.
16. Griffiths CT, Parker LM, Fuller AF: Role of cytoreductive surgical treatment in the management of advanced ovarian cancer. Cancer treat Rep 63:235, 1979.
17. Day TG Jr, Smith JP: Diagnosis and staging of ovarian cancer. Semin Oncol 2:217–222, 1975.
18. Smith JP, Day TG Jr: Review of ovarian cancer at the university of Texas Systems Cancer Center, M.D. Anderson Hospital and Tumor Institute. Am J Obstet Gynecol 135:984, 1979.
19. Hudson CN, Chir M: Surgical treatment of ovarian cancer. Gynecol Oncol 1:370–378, 1973.
20. Hacker NF, Berek JS, Lagasse LD: Gastrointestinal surgery in gynecologic oncology. In Gynecologic Oncology. Edited by Knapp RC, Berkowitz RS. Macmillan and Co., New York, NY. In press.
21. Berek JS, Hacker NF, Lagasse LD, *et al.*: Lower urinary tract resection as part of cytoreductive surgery for ovarian cancer. Gynecol Oncol 13:87, 1982.
22. Hacker NF, Berek JS, Lagasse LD, Nieberg RK, Elashoff RM: Primary cytoreductive surgery for epithelial ovarian cancer. Obstet Gynecol 61:413–420, 1983.
23. Wharton JT, Herson J: Surgery for common epithelial tumors of the ovary. Cancer 48:582, 1981.
24. Hansen MB, Powell DE, Donaldson ES, *et al.*: Treatment of epithelial ovarian carcinoma by surgical debulking followed by single alkylating agent chemtherapy. Gynecol Oncol 10:337, 1980.
25. Stehman FB, Ehrlich LH, Einhorn LH, Williams SD, Roth LM: Long term follow-up and survival in Stage III-IV epithelial ovarian cancer treated with cis-dichlorodiamine platinum, adriamycin and cyclophosphamide (PAC). Proc Amer Soc Clin Oncol. Abstract C-573, 1983.
26. Wharton JT, Rutledge F, Smith JP, *et al.*: Hexamethylamine: An evaluation of its role in the treatment of ovarian cancer. Am J Obstet Gynecol 133:833, 1979.
27. Young RC, Chabner BA, Hubbard SP, *et al.*: Advanced ovarian adenocarcinoma. N Engl J Med 299:1261, 1978.
28. Schwartz PE, Smith JP: Second-look operations in ovarian cancer. Obstet Gynecol 138:1124–1130, 1980.
29. Greco FA, Julian CG, Richardson Rl, *et al.*: Advanced ovarian cancer: Brief intensive combination chemotherapy and second-look operation. Obstet Gynecol 58:199, 1981.
30. Ehrlich CE, Einhorn L, Williams SD, Morgan J: Chemotherapy for Stage III-IV epithelial ovarian cancer with cis-dichlorodiaminoplatinum (11), adriamycin, and cyclophosphamide: A preliminary report. Cancer Treat Rep 63:281, 1979.
31. Edwards CL, Herson J, Gershenson DM, Copeland LJ, Wharton JT: A prospective randomized clinical trial or melphelan and cis-platinum versus hexamethylmelamine, adriamycin and cyclophosphamide in advanced ovarian cancer. Gynecol Oncol 15:261, 1983.
32. Castaldo TW, Petrilli ES, Ballon SC, *et al.*: Intestinal operations in patients with ovarian carcinoma. Am J Obstet Gynecol 139:80, 1981.
33. Blythe JG, Wahl TP: Debulking surgery: Does it increase the quality of survival. Gynecol Oncol 14:396, 1982.
34. Philips BP, Buchsbaum HJ, Lifshitz S: Re-exploration after treatment for ovarian carcinoma. Gynecol Oncol 8:339, 1979.
35. Raju KS, McKinna JA, Barker GH, Wiltshaw E, Jones JM: Second-look operations in the planned management of advanced ovarian carcinoma. Am J Obstet Gynecol 144:650, 1982.
36. Berek JS, Hacker NF, Lagasse LD, Nieberg RK, Elashoff RM: Survival of patients following secondary cytoreductive surgery in ovarian cancer. Obstet Gynecol 61:189, 1983.

5. Staging and second-look operations in ovarian cancer

JONATHAN S. BEREK and NEVILLE F. HACKER

Introduction

The appropriate management of patients with ovarian cancer requires a thorough primary surgical staging, and a subsequent surgical evaluation of response to therapy is often indicated. In most patients, even those whose tumors have disseminated throughout the peritoneal cavity, the extent of disease prior to initiation of therapy is unclear because the metastases are asymptomatic. In addition, since the majority of individuals are in clinical remission at the completion of their chemotherapy, an operative method is needed to assess the response to therapy, because noninvasive techniques are too insensitive. The 'second-look' operation has become a standard procedure in many institutions.

Staging

Ovarian cancer, unlike the other gynecologic malignancies, is a surgically staged disease. Surgical staging permits the identification of those patients with microscopically inapparent dissemination so that more appropriate therapy can be recommended. Patients who have no evidence of metastatic disease are candidates for less intensive therapy than those individuals with documented metastasis. Conservation of reproductive tissues is an important issue in young patients, the majority of whom will have germ cell tumors of the ovary. In appropriately selected patients, contralateral ovaries can be preserved with or without omission of chemotherapy. In addition, comparison to various treatment modalities are only valid if accurate surgical staging data are available.

While the majority of women who present with epithelial carcinomas have metastasis, 30 to 40% have disease confined to the pelvis [1]. Conversely, patients with germ cell malignancies, mostly women under 30 years

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Table 1. F.I.G.O. staging of carcinoma of the ovary

Staging is based on findings at clinical examination and surgical exploration. The final histologic findings (and cytologic, when required) after surgery are to be considered in the staging.

Stage I	Growth limited to the ovaries.	
	Stage IA	Growth limited to one ovary; no ascites
		i. No tumor on the external surface; capsule intact
		ii. Tumor present on the external surface, or capsule(s) ruptured, or both
	Stage IB	Growth limited to both ovaries; no ascites
		i. No tumor on the external surface; capsule intact
		ii. Tumor present on the external surface, or capsule(s) ruptured, or both
	Stage IC	Tumor either Stage IA or Stage IB, but with ascites* present or with positive peritoneal ashings
Stage II	Growth involving one or both ovaries with pelvic extension	
	Stage IIA	Extension and/or metastases to the uterus and/or tubes
	Stage IIB	Extension to other pelvic tissues
	Stage IIC	Tumor either Stage IIA or Stage IIB, but with ascites present or with positive peritoneal washings
Stage III	Growth involving one or both ovaries with intraperitoneal metastases outside the pelvis, or positive retroperitoneal nodes, or both. Tumor limited to the true pelvis with histologically proven malignant extension to small bowel or omentum.	
Stage IV	Growth involving one or both ovaries with distant metastases. If pleural effusion is present, there must be positive cytology to allot a case to Stage IV. Parenchymal liver metastases equals Stage IV.	
Special category	Unexplored cases that are though to be ovarian carcinoma	

The Cancer Committee endorses the histologic typing of ovarian tymors as presented in WHO Publication No. 9, 1973, and recommends that all ovarian epithelial tumors be subdivided according to a simplified version of this. The types recommended at the present time are as follows: serious tumors, mucinous tumors, endometrioid tumors, clear cell (mesonephroid) tumors, undifferentiated tumors, and unclassified tumors.

After separation of cases according to histologic types, the groups shall be staged using above classification.

* Ascites is peritoneal effusion which, in the opinion of the surgeon, is pathologic, or clearly exceeds normal amounts, or both.

of age, present with disease apparently confined to the ovaries or pelvic viscera in 60 to 70% of cases [2]. In patients without macroscopic dissemination, evidence of microscopic disease spread must be sought.

Ovarian cancer is staged according to the International Federation of

Obstetrics and Gynecology (F.I.G.O.), which is listed in Table 1. Ovarian cancer disseminates by exfoliation of tumor cells into the peritoneal cavity, through the retroperitoneal lymphatics, and hematogenously to distant extraperitoneal sites [3]. Thus, the staging of patients with early ovarian carcinoma involves the evaluation of all of these potential sites of dissemination.

In the past, it has been noted that apparent Stage I carcinoma of the ovary has recurred in up to 30 to 40% of patients within 5 years [4]. Patients with apparent Stage II carcinoma of ovaries, i.e. with spread to the tubes, uterine serosa, pelvic peritoneum, or with positive ascites, have had a 40 to 50% relapse rate over 5 years. Presumably some of these relapses relate to the fact that the tumor has disseminated at the time of discovery, and microscopic metastases were not documented in many of these patients; while others had tumors which were insensitive to primary therapy.

Preoperative assessment

In most patients, the diagnosis of an ovarian malignancy is made at the time of exploratory laparotomy, often as an unexpected or incidental finding.

In a young patient (i.e., under 30 years of age), most malignancies are of germ cell origin. If a pelvic neoplasm is suspected, a pelvic ultrasound may provide a clue that the malignancy is present, as a complex echogenic mass or a multilocular structure suggests a neoplasm. Persistent adnexal masses of 8 centimeters in diameter or greater which do not decrease in size on hormonal suppression should be surgically evaluated. Serum markers utilizing the beta-subunit of human chorionic gonadotropin (B-HCG) or alpha-fetoprotein (AFP) are important to obtain, as this might be the most reliable measure of residual tumor. The serum should be assayed for these markers pre-operatively whenever surgery is undertaken for a presumed adnexal neoplasm.

In older patients, especially post-menopausal women, a palpable adnexal mass must be assumed to be malignant neoplasm until proven otherwise.

In addition to the routine laboratory tests, the evaluation should include a chest x-ray, and if there is a suspicious lesion, lung tomography or a chest CT scan. A barium enema should be performed routinely in women over 40 years, and a upper gastrointestinal series should be performed if there are any signs or symptoms of a small bowel obstruction. In addition, if there are any suspicious breast masses, mammography should be obtained. An intravenous pyelogram can rule out a pelvic kidney. The performance of a liver/splen scan, abdominal CT scan, or lymphangiography is optional, since surgical evaluation of these structures is more sensitive. Pelvic ultrasonogra-

phy or CT scan may be useful to differentiate between lesions of non-gynecologie origin.

Preoperative preparation should include a low mechanical bowel cleansing, and preoperative prophylactic antibiotics and low dose subcutaneous heparin in older patients.

Technique of surgical staging laparotomy

If malignancy is suspected, it is important to perform a either a midline or paramedian vertical incision in the abdomen. This allows adequate exposure so that the upper abdominal viscera can be adequately inspected, palpated and sampled.

In the event that an unexpected malignancy is discovered when a transverse abdominal incision has been performed, greater access to the abdominal viscera can be accomplished by dividing the lower rectus muscles in a transverse direction using electrocautery. The inferior epigastric arteries and veins must be isolated, ligated, and divided during this process. In order to sample the periaortic lymph nodes and perform an omental biopsy, occasionally the incision must be extended laterally, using a 'hockey stick' technique.

Upon entering the abdomen and the discovery that there is a potential for cancer confined to the ovary or pelvis, multiple peritoneal cytologies should be obtained. If any free fluid is present, it should be collected and submitted for cytologic evaluation. In addition, we recommend the performance of 5 separate cytologic assays: the pelvis cul-de-sac, both paracolic gutters, and both hemidiaphragms, submitted separately in order to maximize the probability of detecting exfoliated malignant cells. The cytologic washings should be performed using 50 to 100 cc of normal saline instilled and recovered from each of the separate locations. In the diaphragm areas, the collection of fluid is often facilitated by the use of a rubber catheter connected to a syringe. The tip of the catheter can be placed in its desired location so that the fluid can be instilled and aspirated without having to visualize the site.

The entire intra-abdominal contents must be carefully inspected and palpated. A systematic approach should be employed so that the liver, both kidneys, spleen, and lesser omentum are examined. The periaortic area should be carefully palpated for the presence of any adenopathy, which if present, would require biopsy. The entire length of the small intestine, from the ligament of Trietz to the cecum, along with its mesentery should be palpated. The entire colon should likewise be examined. Both halves of the diaphragm should be carefully palpated. Inspection of this region is often facilitated by the use of a laparoscope, especially to direct a biopsy of any

small, suspicious lesion. This will permit satisfactory magnification of this area which is often somewhat difficult to visualize. In any case, the cytologic evaluation of the diaphragm should be obtained by performing a pap smear of the right side.

In the event that there is no macroscopic evidence of disease dissemination, multiple intraperitoneal biopsies are performed. All sites of potential dissemination are sampled. We routinely sample the peritoneum of the pelvic cul-de-sac, both paracolic gutters, peritoneum of the small bowel mesentery, bladder and pelvic sidewalls, and the infundibulopelvic ligament.

An infracolic omentectomy is performed by ligating the vessels along the transverse colon. For staging purposes, it is not necessary to remove the gastrocolic ligament if the omentum is palpably normal.

When feasible, a pelvic and periaortic lymphadenectomy below the level of the renal vessels should be performed. The procedure should include the external iliac lymph nodes, the hypogastric nodes, bilateral common iliac nodes, and periaortic nodes.

If the contralateral ovary is grossly normal, it should be bivalved to rule out occult malignancy. If there is no evidence of contralateral malignancy on frozen section in a patient with a probable Stage I germ cell malignancy or a unilateral neoplasm of low malignant potential, it can be preserved until definitive pathology is available.

Epithelial malignancies

Patients with a Stage IAi, grade 1 tumor have been shown to have at least a 90% 5-year survival, regardless of whether single alkylating agent therapy (melphelan), pelvic radiation therapy, or no therapy is used in these patients [5]. Thus, a well-differentiated lesion confined to one well-encapsulated ovary with negative intraperitoneal cytology does not require any

Table 2. Comparison of recurrences by grade and treatment of patients with stage I epithelial cancer

	Grade					
	1		2		3	
Therapy	No.	%	No.	%	No.	%
No therapy	1	6	2	28	2	40
Radiation therapy	4	29	2	40	1	25
Chemotherapy	1	4	0	—	1	17

Ref. Hreschchyshun et al. (1980) [5].

additional therapy. Indeed, the recurrences of surgical Stage I patients with grade 1 lesions is not decreased by the addition of pelvic radiation and not significantly lessened by the use of an alkylating agent (Table 2), while patients with grade 2 and 3 cancers appear to benefit from alkylating agent therapy. Clearly, the recurrence rate is also related to subsets of Stage I, as outlined in Table 3. Also, patients who have non-metastatic neoplasms of low malignant potential, so-called 'borderline' neoplasms of the ovary, do not require further therapy; and the need for therapy in disseminated lesions of low malignant potential is controversial and under investigation.

Metastasis in apparent Stage I and II ovarian cancer have been reported by several authors [6–13] (Table 4). When summarized, these reports document occult metastasis in almost 30% of analyzed cytologic specimens, 7% of the diaphragm biopsies, 7.5% in the aortic lymph nodes, 3.3% in pelvic lymph nodes, and less than 3% in the omentum. Thus, at least 30% of patients without apparent metastasis have already developed occult tumor spread. Recently, a prospective study by the Gynecologic Oncology Group [11] showed a 3.2% incidence of pelvic nodes and 5.5% of periaortic nodes positive in Stage I and II ovarian cancer. In that series, diaphragm metastases were documented in 1% of patients, and omental metastases in 2%. Musumeci [14] recently reported a series of 365 patients who under-

Table 3. Relationship of tumor stage and recurrences

		Recurrence	
Stage	No. of Patients	No.	%
IA(1)	48	5	10
IA(2)	28	5	18
IB(1)	4	1	25
IB(2)	6	3	50

Ref. Hreschchyshyn et al. (1980) [5].

Table 4. Metastases in apparent stage I and II ovarian cancer

Location	Patient	No. positive	%
Diaphragm	169	12	7.1
Aortic lymph node	158	12	7.5
Pelvic lymph node	93	3	3.3
Omentum	134	3	2.2
Cytology	87	26	29.9

Ref. [6–13].

went lymphangiography and staging of ovarian cancer. Eighty-seven of these patients were apparent Stage I and II, and 8 of these (9.2%) had positive pelvic and/or periaortic lymph nodes.

Germ cell tumors

Dysgerminomas, endodermal sinus tumors, and immature teratomas have a tendency to spread early via the retroperitoneal lymphatics [2, 3]. These patients, therefore, should undergo pelvic and periaortic lymphadenectomy, because as high as 20 to 30% of patients with lesions apparently confined to the pelvis have lymph node metastases [2].

Bilaterality is extremely rare for endodermal sinus tumors and thus the contralateral ovary can usually be preserved. The rate of bilateral dysgerminomas is 5 to 10% and bilateral immature teratomas, approximately 10% [2]. Thus, it is recommended that the contralateral ovary undergo a wedge biopsy and if this is normal at frozen section, the ovary can be preserved. It is important to preserve reproductive function in these patients whenever possible, since most are young and of low parity. In well-staged patients with unilateral ovarian germ cell tumors, the uterus and contralateral adnexa should be preserved. If a frozen section diagnosis is equivocal, the reproductive tissue should be preserved, since tissues which are found to be malignant on permanent section can be extirpated at a subsequent procedure.

Laparoscopy

The laparoscope has been used by several authors [15–21] for the initial staging of patients with ovarian cancer (Table 1). The majority of these patients had initial laparotomy prior to referral for chemotherapy, bur further surgical evaluation was considered advisable because formal surgical staging had either not been carried out or been performed inadequately.

Operative technique of laparoscopy

Laparoscopy is performed using a 12 mm Wolf laparoscope but is significantly more complicated than routine laparoscopy because of the risk of bowel injury [22]. In order to minimize the morbidity, the 'open' laparoscope as described by Hassan [23], or a needle laparoscope as described by Berek *et al.* [22], should be employed prior to the introduction of the large-bore laparoscope in order to avoid bowel perforation in this group of patients who typically have multiple intraperitoneal adhesions.

The 'open' laparoscope is actually a laparoscopic sheath which is sutured to the fascia. The surgeon makes a 2 to 3 cm vertical skin incision just below the umbilicus. The subcutaneous tissue and the linea alba of the rectus abdominus fascial sheath are incised. The peritoneum is identified and carefully entered in order to avoid adherent small intestine. If bowel is adherent, gentle adhesion lysis is performed to create an opening into the peritoneal cavity. A 'purse string' suture is placed on the peritoneum to seal the sheath. The open laparoscopic sheath is then sutured to the fascia and the laparoscope inserted through this sheath [15, 23].

Alternatively, the needle laparoscope, which is a 14 gauge fiberoptic endoscope (1.7 mm diameter) can be passed through a 12 gauge needle trocar [21]. This instrument is approximately the same caliber as the needle. If one plans to use the needle laparoscope, the Verres needle is inserted under the umbilicus by picking up the skin and subcutaneous tissues bilaterally in order to allow the bowel to fall free from the anterior abdominal wall. After the peritoneal cavity is maximally distended with 3 to 4 liters of gas, the needle laparoscope is inserted at the right rectus border halfway between the umbilicus and the symphysis pubis. In this manner, the entry site of the Verres needle is identified and a suitable location is selected for the insertion of the large-bore trocar. A secondary trocar can also be inserted through the needle laparoscope site, so that one can sequentially pass a manipulation probe instrument, adhesion lysis scissors, a fluid introducer and aspiration tube, as well as various punch biopsy instruments [15].

The large-bore laparoscope in place, a survey of the peritoneal cavity is performed. The pelvis is identified first, and the cul-de-sac must be thoroughly inspected. Using a surgical gauze on a sponge forceps place in the vagina or a Hummie canula in the cervix, if still present, the vagina can be elevated to improve visualization. Lysis of intraperitoneal adhesions can be performed with a scissors instrument inserted through the secondary trocar. The pericolic gutters, the omentum if present, both hemidiaphragms, the liver, stomach, and the serosal surfaces of the small intestine, colon and their mesenteries should be visualized. It is important to perform laparoscopy on a surgical table which an be easily tilted and pitched so that the patient's position can be manipulated. In this manner, the amount of surface area that can be evaluated is maximized [15].

If free fluid is present in the abdominal cavity, it should be aspirated and submitted for cytologic evaluation. Small amounts of fluid are often present in the cul-de-sac. Following this, 50 to 100 ml of normal saline is instilled via fluid instillation tube connected to a 50 ml syringe. The fluid is first instilled into the pelvic cul-de-sac and collected. A similar volume of fluid is instilled into each pericolic gutter and right diaphragm, aspirated, and submitted separately for cytologic evaluation [15].

In patients with no gross evidence of disease, multiple intraperitoneal

biopsies should be performed, including representative biopsies of each major area in the peritoneal cavity. Any suspicious areas on the visceral and parietal peritoneum are biopsied, as well as peritoneal elevations and sites of known previous tumor. A biopsy of the diaphragm can be readily accomplished and lesions as small as 0.1 cm can be successfully removed. Occasionally the biopsy produces a small amount of bleeding, but this can be controlled using electrocautery which is an integral part of the biopsy instrument [15, 22].

The laparoscope for initial staging

Use of the laparoscope in the surgical staging of ovarian cancer is summarized in Table 5 [15–21]. The initial study by Bagley *et al.* [23], reported that of 16 patients, 7 (44%) had detectable diaphragmatic metastasis had pretreatment laparoscopy following laparotomies in patients who were thought to have disease confined to the pelvis. This observation suggested that occult metastases were more frequent than previously realized and that the laparoscope could play a role in the initial staging of ovarian cancer.

There have been reports of 210 patients undergoing laparoscopy for initial staging (Table 5). Of these, either biopsy or cytologic evidence of more advanced disease was found 110 (52%) patients. Significant complications occurred in less than 3% of patients. Metastases were documented primarily either by detected disease on the diaphragm or by obtaining positive intraperitoneal cytologic evaluations.

These reports indicate the importance of performing a thorough initial staging operation. Since over one-half of patients referred to these centers have evidence of metastatic disease after only a brief interval (typically only several weeks), one can presume that most of the patients had disease at the time of initial laparotomy which went undetected. Most of the patients had

Table 5. Laparoscopy for initial staging of ovarian cancer

Author	Year	Patients	No. positive	Complications
Ozols et al. [21]	1981	83	42 (51%)	3%
Mangioni et al. [20]	1979	23	11 (48%)	0
Piver et al. [19]	1978	31	8 (26%)	0
Spinelli et al. [18]	1976	27	19 (71%)	2%
Rosenoff et al. [19]	1975	30	23 (77%)	0
Bagley et al. [16]	1973	16	7 (44%)	2%
Total		210	110 (52%)	0–3%

Ref. [15].

not had omental biopsies or cytologic evaluation of the peritoneal surfaces, or an adequate evaluation of the retroperitoneal lymph nodes at the time of initial therapy [21].

Comparison of pre-treatment laparotomy to non-invasive techniques

Pre-treatment or 'initial staging' laparoscopy has been compared to non-invasive testing, principally radiographic imaging techniques (Table 6). In one series [21], while 45% of the patients had either a pelvic mass or clinically detectable ascites, scans and radiograms of the bowel or chest were positive in only 20 to 40%. Lymphangiography was positive or suspicious in about 40% of patients evaluated. These tests are therefore an insensitive determinant of disease, even in many patients with gross evidence of tumor. In those who have no clinically detectable evidence of disease, only a rare patient has an abnormal pretreatment non-invasive test. In this series, 56 of 88 patients had disease documented at pre-treatment laparoscopy, 42 of whom had negative non-invasive studies. This comparison indicated that laparoscopy is a more sensitive means of documenting disease pre-treatment than non-invasive tests. However, since non-invasive testing added no more information than the physical examination, this comparison alone does not provide a reliable indication of the adequacy of laparoscopy for the initial staging of ovarian cancer.

The laparoscope is intermediate in utility between disease detected by physical examination and noninvasive testing, and the performance of a laparotomy. The laparoscope does not permit detection of occult metastasis in one-fourth to one-third of patients prior to therapy, principally as a result

Table 6. Comparison of non-invasive evaluation of patients with ovarian cancer to laparscopic findings

Examination	No. performed	Abnormal	%
Physical	88		100
Ascites		17	19
Mass		23	26
Nucleotide scans	61	16	26
Chest X-ray	88	20	23
Lymphangiogram	73	29	41
BaE, UGI, IVP	43	16	40
Ultrasound	13	4	31
Residual disease detected		56	64
Undetected by non-invasive study		42	48

Adapted from Ozols et al. (1981) [21].

of failure to adequately visualize the entire peritoneal cavity, or to evaluate the retroperitoneal lymph nodes. Furthermore, in the presence of a positive laparoscopy, disease cannot be adequately resected. Since the performance of an initial optimal cytoreductive operation prior to the initiation of therapy in patients with epithelial tumors is a significant variable with regards to subsequent survival, a thorough primary exploration is necessary [24–26].

Based on this analysis, it is the author's recommendation that the laparoscope should be used for initial staging only in a patient who is referred for laparotomy and who has had apparent inadequate primary staging *and* refuses another laparotomy.

Second-look operations

Since there is no reliable serum assay or x-ray test available to monitor the amount of residual disease in the majority of patients with ovarian cancer, second-look operations are often performed in those who are clinically free of disease at the completion of their chemotherapy.

The definition of secondary laparotomies for ovarian cancers has not been standardized. The term 'second-look' has been used to describe secondary laparotomy performed on patients with ovarian cancer for a variety of reasons, including operations for tumor resection in patients with obvious disease progression or bowel obstruction [27–35]. Since the intent of the procedure is to evaluate response to therapy in those patients who are otherwise unevaluable, we recommend that the term be employed only for those operations performed on patients who are clinically free of disease following therapy.

Technique of a second-look laparotomy

A negative second-look laparotomy is defined as one in which there is no visible, microscopic or cytologic evidence of tumor at the time of surgery. When no macroscopic disease is discovered, a thorough search for occult disease is undertaken as outlined below. If any single biopsy or cytologic specimen reveals evidence of malignancy, the procedure is termed positive.

The technique of second-look laparotomy is essentially the same as that described for a staging laparotomy. The patient is placed in a supine position, and a midline or paramedian vertical incision is made to gain adequate exposure to the upper abdomen. If no macroscopic disease is visualized, a thorough and systematic evaluation of the intra-abdominal contents is per-

formed. All intra-abdominal viscera are carefully palpated for any evidence of disease. Visualization of the diaphragm can be facilitated by the insertion of a laparoscope through the incision, which can permit the detection of a small (millimeter) lesions. A directed biopsy of this area may permit the identification of disease which could be overlooked in a random sample. A Papanicolaou smear of the right hemidiaphragm is performed for cytologic evaluation.

Multiple intraperitoneal cytologies and biopsies should be performed in the manner identical to the technique of a staging laparotomy. Any free peritoneal fluid should be submitted separately prior to the performance of peritoneal washings.

Multiple separate biopsies of the peritoneal surfaces are taken at any site of previously documented tumor, the pedicles of the infundibulo-pelvic ligament, the pelvic cul-de-sac, the bladder dome, peritoneum, the pelvic sidewalls, the pericolic gutters, and any irregular surface elevations or suspicious areas on the bowel and the peritoneal surfaces should also be submitted for histopathologic evaluation. A minimum of 20 to 30 such biopsies should be taken.

A pelvic and periaortic lymph node dissection below the level of the renal vessel should be performed in patients in whom these tissues have not been removed, as is the case with most undergoing treatment for Stage III disease. Residual omentum should be removed from the greater curvature of the stomach.

In the event that macroscopic tumor is discovered, it is our policy to proceed to attempt a secondary cytoreductive surgery if optimal resection is considered feasible, since significantly prolonged survival can be seen in patients in whom optimal secondary resection of tumor can be accomplished (36). (See Chapter 4, Cytoreductive Surgery).

Results

Previous reports have concentrated on patients survival based on findings at second-look surgery [27–34]. These reports indicate that a procedure during which there is no histopathologic or cytopathologic evidence of disease present is associated with a significantly prolonged survival as compared to those patients that have such disease documented. Unfortunately, relapses following these negative examinations have been reported from 20 to 50% over the subsequent 4 to 5 year interval. Patients with microcopic disease only have been shown to have an intermediate survival between these 2 groups [34].

There has been inconsistency in the evaluation of data from prior analysis, since various definitions of second-look laparotomy have been em-

Table 7. Second-look Laparotomy: Sites of microscopic disease in patients with visible evidence of tumor

Patient	Peritoneum	Diaphragm	Nodes	Cytology
1	+	+	–	+
2	+	–	Pelvic	+
3	+	–	Pelvic	–
4	–	–	Pelvic	–
5	–	–	Aortic	–
6	–	–	Pelvic and aortic	–
7	–	–	–	+
8	–	–	–	+
Total	3 (37.5%)	1 (12.5%)	5 (62.5%)	4 (50%)

Ref. [35].

ployed. While some authors have restricted the definition to those patients undergoing laparotomy who are clinically free of disease [30, 31] others have included patients who have clinically appreciable evidence of progression [27–29, 32, 33] (Table 7). In the largest single series, Schwartz and Smith [27] assess the response to chemotherapy in 186 patients with epithelial tumors of the ovary. Fifty-eight patients (31%) had no evidence of disease detected, and their chemotherapy was discontinued. In this group of patients, the 5-year survival was 72%. However, only 73 of the 128 (57%) with Stage III and IV disease were clinically free at the completion of their therapy. However, the majority of negative second-look surgeries were documented in patients originally staged as I or II (32 of 58, 55%), whereas only 26 of 128 patients (20%) with Stages III and IV disease were negative. Thus, 26 of 73 (36%) of patients with Stage III and IV disease who were clinically free of disease had a negative scond-look.

Tumor grade was noted by Webb *et al.* [31] to be correlated with a negative second look among 71% of patients with grade 1 tumors having a negative second look 57% grade 2, 47% of grade 3, and 22% of grade 4. However, in the series by Schwartz and Smith [27], tumor grade was not a significant predictor of second look findings. In series by Berek *et al.* [35], tumor grade was highly correlated with the finding of a negative second look laparotomy in that the majority who had a second look had not had either a grade 1 or 2 tumor (17 of 37 patients, 46.0%), as opposed to only 1 of 19 patients (5.3%) with grade 3 or 4 carcinomas. Tumor grade has not been analyzed in the majority of reports.

Because most previous studies have utilized patients who had received heterogenous treatments, numbers have been insufficient to evaluate the influence of any type of therapy and another. In a series by Smith [28],

patients treated with Melphalan alone had a significantly higher number of negative second-looks and prolonged survival compared to those patients treated with pelvic radiotherapy alone. However, 5-year survivors in these patients with a positive second-look were few (30% and 14% respectively). In the series by Berek *et al.* [35] the use of at least 6 cycles of cis-platinum, doxorubicin, and cyclophosphamide was associated with a significantly greater percentage of negative second-look operations (15 of 28, 53.6%), compared to those treated with doxorubicin and cyclophosphamide (2 of 17, 11.8%), and compared to those treated with melphalan alone (1 of 11, 9.1%) ($P < .01$).

The amount or residual tumor at the completion of primary surgery is a strong predictor of outcome in several reports [27, 29–31, 33, 35] and not significant in another [32]. The extent of cytoreduction *per se* of metastatic disease appears to be associated with the greater probability of a negative second look in those patients whose largest metastatic tumors are between 1.5 and 10 cm in diameter and reduced to optimal (< 1.5 cm) [35]. The size of the primary lesion, however, does not appear to influence the likelihood of a negative second-look. Patients with very large diameter of metastatic disease (> 10 cm) have a low probability of a negative second-look regardless of whether these tumors are resected. Indeed, the probability of a negative second-look is the same for patients whose metastatic tumors are small (< 1.5 cm) at the time of initial laparotomy, and for those whose metastatic lesions are 1.5 to 10 cm were reduced to < 1.5 cm. Since the tumor burden at the completion of the primary surgery is predictive of outcome, the performance of optimal cytoreductive surgery on patients with metastasis between 1.5 to 10 cm appears to improve the probability of achieving a pathologic remission documented by second-look. This finding is in accord with those reported to be associated with survival following primary cytoreductive surgery [26].

The number of cycles of chemotherapy to be utilized in patients with ovarian cancer is still being defined. In an earlier report by Smith *et al.* [28], it was indicated that survival was prolonged in those patients treated with 10 or more cycles of Alkeran compared to those treated with fewer cycles. Due to the concern for the development of leukemia following prolonged use of alkylating agent therapy, there has been a tendency to utilize the shorter courses of chemotherapy [37–39]. Indeed, as a result of cumulative toxicities of combination therapies, it is not practical to utilize more than 10 or 12 cycles. In our experience, the probability of achieving a negative second-look laparotomy is the same for those patients receiving 6 to 9 cycles of combination therapy, as opposed to 10 or more cycles [35]. These data indicate that epithelial carcinomas are responsive to these combinants of cytotoxic agents exhibit sensitivity early in their course of treatment, similar to the experience with metastatic germ cell tumors of the ovary. When the

extent of residual diseases is considered, the use of fewer than 10 cycles of chemotherapy is appropriate, i.e., the proportion of optimal patients does not influence outcome when corrected for the number of cycles. Indeed, the recurrence rate is not related to the number of cycles of chemotherapy, since additional cycles of therapy following 6 to 9 cycles in epithelial tumors did not decrease the probability of recurrence.

A thorough second-look operation is important. A significant proportion of patients without macroscopic tumor have microscopic disease documented in less than 10% of biopsies and cytologies taken, and therefore a large number of specimens must be submitted for evaluation. Since as many as 30% of patients without macroscopic disease have lymph node metastases detected in pelvic or periaortic lymph nodes, the performance of a lymph node dissection appears to be indicated [35]. These data may help to explain the 30 to 50% relapse rate over 5 years reported in an earlier series following reported negative second-look operations. In a series of 8 patients with apparently no macroscopic evidence of tumor, lymph nodes were positive in 5, and cytology was noted to be positive in 4 (Table 7).

Second-look laparoscopy

Laparoscopy for second-look evaluation for patients with ovarian cancer has been reported by several authors [17, 18, 20–22, 40–42] (Table 8). Rosenoff *et al.* [17] first reported that 39% (7 of 18 patients) who were in clinical remission had evidence of tumor at laparoscopy. The percentage of positive laparoscopies in subsequent reports has varied from 11 to 50%.

The advantage of the laparoscope, of course, is that it is a more limited

Table 8. Laparoscopy for second-look in patients with ovarian cancer

Author	Year	No. procedures	No. positive	% positive
Berek et al. [22]	1981	119	41	34
Ozols et al. [21]	1981	66	33	50
Piver et al. [40]	1980	22	8	36
Spinelli et al. [18]	1979	42	11	23
Mangioni et al. [20]	1979	34	5	11
Lacey et al. [41]	1978	19	5	26
Smith et al. [42]	1977	24	7	29
Rosenoff et al [17]	1975	18	7	39
Total		344	117	34

Ref. [15].

operative procedure than the laparotomy. However, the information furnished by the laparoscope is also more limited, especially because of its inability to evaluate the retroperitoneal lymph nodes. In the presence of extensive adhesions in the peritoneal cavity, visualization is hampered particularly in the pelvic cul-de-sac and under the diaphragm, two frequent sites of malignancy [15].

Operative technique for second-look laparoscopy

The technique used for second-look is identical with that when the laparoscope is used for staging purposes, as outlined above. It is important to perform as thorough a laparoscopy as possible, which is facilitated by the use of a second puncture site through which multiple instruments can be inserted, including probe, scissors, biopsy and cauterization tools. The abdomen should be maximally distended following adequate endotracheal intubation for maximum respiratory control, which helps to outline areas of adhesions which can be lysed with the lysis instrument. Occasionally a third puncture site must be utilized to gain better access to the upper abdomen.

Results

Adequate laparoscopy is defined as the surgeon being able to visualize the entire peritoneal cavity from the cul-de-sac to the diaphragm, including the pericolic gutters and as much bowel serosa as is made possible by probe manipulation, without a significant complication. Defined in this manner, laparoscopy is reported to be successful in about two-thirds of the three-fourths attempted procedures [15].

Intraperitoneal biopsies and cytologic evaluations performed in conjunction with the direct visualization of peritoneal cavity are as outlines above. Important diagnostic adjuncts may significantly improve the accuracy of the operation. In our experience, random biopsies or cytologic washings are positive in 4 out of 46 (9%) patients where visualization of peritoneal surfaces revealed no suspicious lesions [22]. In a series by Piver *et al.* [40], 4 out of 22 (18%) of the patients have positive cytologic washings as the only evidence of persistent disease. In another study, Mangioni *et al.* [20], 19 patients (32%) demonstrated positive cytology with tumor who could no otherwise be visualized. The unexpected finding of microscopic disease in some patients suggests the failure of laparoscopy to detect occult disease that could result from the inadequate intraperitoneal biopsies.

In a study of 57 patients [22], laparoscopies performed after 6 months of

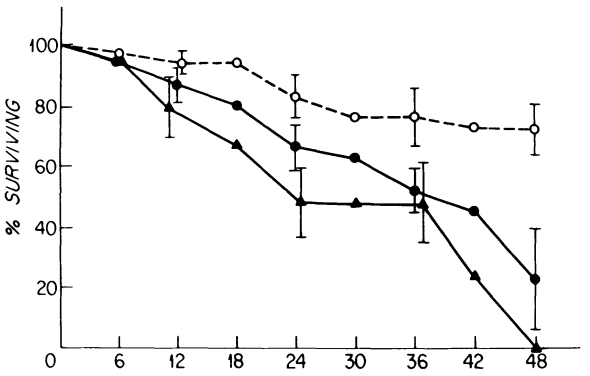

Figure 1. Actuarial survival of patients following successful laparoscopy 6 months after initiation of therapy, comparing the patients with negative and positive findings to the entire patient population. Vertical bars represent 95% confidence limits. Open circles, broken line = negative procedure ($N = 37$); solid circles, solid line = all procedures ($N = 57$); solid triangles, solid line = positive procedure ($N = 20$).

chemotherapy for patients with epithelial malignancies of the ovary were negative in 37 patients and positive in 20. The survival of these patients subsequent to their laparoscopies is presented in Figure 1. The top curve represents the survival of those patients with negative laparoscopies, and the lower curve is the survival of those patients with no evidence of clinical disease, but who had gross subclinical and microscopic disease discovered at laparoscopy. There is a statistically significant curve between 6 and 48 months, a difference which also exists if one excludes patients with Stage I and II disease.

Following the initial laparoscopy, the probability of disease status changing at subsequent operations is presented in Table 9. The likelihood of recurrence can be related to the number of laparoscopies performed at 6-month intervals and the duration of clinical remission. Eleven of the 37 patients had initially negative laparoscopies and developed evidence of

Table 9. Probability of recurrence following consecutive negative laparoscopy

No. of negative laparoscopies	Months	Mean duration of remission	Probability of recurrence after (months)				
			12	18	24	36	48
1	6	22	0.08	0.16	0.22	0.30	0.30
2	12	27		0.10	0.16	0.22	0.22
3	18	39			0.08	0.12	0.12
4	24	41				0	0.08
5	30	48					

Ref. Berek, Griffiths, and Leventhal (1981) [22].

recurrence 12 to 40 months later (mean time = 22 months). Thus, the initial negative laparoscopy performed only after 6 months of therapy identified 26 out of 37 patients (70%) who remained without clinical evidence of disease for 3 more years [22].

These facts imply that the sensitivity of the laparoscope is in the range of 70%. If one multiplies this number by the percentage of successful procedures (73% in our experience), the surgeon can be expected to accurately rule out persistent disease in about one-half of those who have an attempted laparoscopy.

The laparoscope has not systematically been studied as a means of detecting persistent disease or evaluating patients with ovarian neoplasms of low malignant potential, which are in patients who have not been subjected to chemotherapy. While the treatment and followup of patients with borderline ovarian neoplasms is controversial, it is our current recommendation that patients undergo a laparoscopic evaluation about 6 months following identification and initial resection of their disease, especially in those patients who have retained contralateral ovaries. This may prove to be useful in patients who desire preservation of reproductive functions and a means of detecting early recurrences.

Since in our experience and in the experience of others there is at least a 20% relapse rate following a negative second-look laparotomy [35], it has been our policy to perform a 'third-look' laparoscopy in approximately 6 to 9 months following their second look procedure. Thus far, out of 12 such laparoscopies performed, we have detected 2 early recurrences, and have been able to initiate second-line therapy with microscopic relapse only. Theoretically, if salvage therapies are to be useful in this disease, they will be useful principally when the disease can be detected prior to macroscopic recurrence.

Complications and morbidity associated with laparoscopy in patients associated with ovarian cancer occur in about 10 to 20% of procedures [15]. Most of these complications are minor, such as superficial wound infections, subcutaneous emphysema, and subcutaneous hematoma. In 2 to 7% of procedures, the complications involve bowel perforations, requiring a laparotomy to repair the defect. In one series [22], the number of complications necessitating laparotomies to repair bowel perforation or to control bleeding in the bowel mesentery was decreased by the use of needle laparoscopy prior to the insertion of the large-bore trocar. The rate of serious complications was reduced from 19% (7/37) prior to the use, to 1.2% (1/82) ($P < .05\%$). A similar reduction of bowel perforation can be also accomplished using the 'open' Hassan laparoscopy equipment. In our series, 87% of patients required only 24 hours of hospitalization, which indicates that the laparoscope is a much less invasive means of evaluating the peritoneal cavity, especially in patients where there is a low risk for recurrence.

Comparison of laparoscopy and laparotomy for second-look

Several authors have compared the findings of laparoscopy with laparotomy by performing the latter procedure immediately following the former. Piver *et al.* [40] studied 10 patients in this manner, and found that 2 of these (20%) had disease discovered at laparotomy after a negative laparoscopy. However, Ozols *et al.* [21] studied 22 patients, and 12 of thse (55%) had disease detected at laparotomy which had not been observed using the laparoscope. Mangioni *et al.* [20] reported 18 patients who underwent exploratory laparotomy immediately after or within 10 days of laparoscopy, and 6 (22%) of these had persistent tumor. Since of these discrepancies may be explained by omission of multiple biopsies and cytologic washings at laparoscopy. However, these reports indicate that a negative laparoscopy is not sufficient to determine whether or not to discontinue therapy in the majority of patients.

Many authors have concluded from these findings that a positive laparoscopy can thus avoid secondary laparotomy in 15 to 40% of patients [15]. This conclusion, however, is based on the assumption that persistent disease documented at secondary laparotomy need not be resected, since it will not influence response to 'second-line' therapy or subsequent survival. At least one report [36] challenges this notion, and thus this point is thus controversial. On our service, almost two-fifths of patients were able to undergo optimal tumor resection at the time of secondary laparotomy, and this group had a median survival of 20 months compared to only 5 months of the suboptimal group ($P<.01$). If second-line therapies become more successful, tumor resection might become more important in those individuals who have surgically resectable tumors found at second-look.

Thus, while the role of the laparoscope for second-look in ovarian cancer is being defined, it is recommended that the technique can be utilized for: 1) an interval evaluation of response during the course of chemotherapy; in particular in those patients in whom toxicity as a result of chemotherapy might require a significant modification of therapy; 2) patients who either refuse laparotomy or are considered medically unfit to undergo laparotomy; 3) the follow up of patients with neoplasms of 'low malignant potential'; or the management of patients with cancer apparently confined to one or both ovaries (Stage I) who have received no adjuvant therapy; however, neither of these issues has been adequately studied as yet; 4) as a third-look procedure to detect early recurrences in patients who have undergone a thorough negative second-look laparotomy.

The laparoscope is contraindicated as a routine means of determining whether or not to discontinue therapy, when the patient has obvious evidence of clinical progression, or a patient who presents with a bowel obstruction. In patients who have received whole abdominal radiation ther-

apy, the laparoscope is relatively contraindicated as the extent of intra-abdominal adhesions often preclude its usage.

Summary

A thorough primary staging laparotomy should be performed in all patients who have ovarian malignancy which microscopically appears to be confined to the pelvis, in order to define those patients with occult metastatic disease. More rational therapy can thus be employed, and presumably a greater number of these patients can be cured.

Following completion of primary chemotherapy, a thorough second-look laparotomy should be performed in order to determine whether to discontinue therapy, or to initiate salvage therapies. Until such time as a blood marker is developed for epithelial tumors of the ovary, second-look laparotomy will play a useful role in further evaluation of therapeutic modalities for the treatment of these diseases.

The laparoscope is a more limited technique, but probably has a useful role, especially in monitoring patients with early response to therapy during combination therapy, or to detect early relapses following a negative second-look laparotomy. Because of its relative ease and low morbidity, it is occasionally useful, but should not be routinely employed to determine when to discontinue therapy.

References

1. Day TG, Smith JP: Diagnosis and staging of ovarian cancer. Semin Oncol 2:217, 1975.
2. Scully RE: Tumors of the ovary and maldeveloped gonads. AFIP Fascicle 16, Washington D.C., 1979.
3. Plentl AA, Friedman EA: Lymphatic system of the female genitalia. W.B. Saunders Co., Philadelphia, 1971.
4. Fisher R, Young R: Advances in the staging and treatment of ovarian cancer. Cancer 39:967, 1977.
5. Hreschchyshyn MM, Park RC, Blessing JA, Norris HJ, Levy D, Lagasse LD, Creasman WT: The role of adjuvant therapy in Stage I ovarian cancer. Am J Obstet Gynecol 138:139, 1980.
6. Piver MS, Barlow JJ, Lele SB: Incidence of subclinical metastasis in Stage I and II ovarian carcinoma. Obstet Gynecol 52:100, 1978.
7. Creasman WT, Rutledge F: The prognostic value of peritoneal cytology in gynecologic malignant disease. Am J Obstet Gynecol 110:773, 1971.
8. Keettel WC, Pixley EE, Buchsbaum HJ: Experience with peritoneal cytology in the management of gynecologic malignancies. Am J Obstet Gynecol 120:174, 1974.
9. Delgado G, Chun B, Caglar H: Paraaortic lymphadenectomy in gynecologic malignancies confined to the pelvis. Obstet Gynecol 50:418, 1977.
10. Morton DG, Moore JG, Chang N: The clinical value of peritoneal lavage for cytologic

evaluation. Am J Obstet Gynecol 81:1115, 1961.
11. Buchsbaum H, Delgado G, Miller A: Surgical staging of ovarian cancer. Gynecologic Oncology Group: Statistical Report. Denver, CO, July 1983, pp 27–28.
12. Knapp RC, Friedman EA: Aortic lymph node metastases in early ovarian cancer. Am J Obstet Gynecol 119:1013–1017, 1974.
13. Rosenoff SH, DeVita VT Jr, Hubbard S, *et al.*: Peritoneoscopy in the staging and followup of ovarian cancer. Semin Oncol 2:223, 1975.
14. Musumeci R, DePalo G, Kenda R. *et al.*: Retroperitoneal metastases from ovarian carcinoma: Reasessment of 365 patients studied with lymphography. Am J Radiol 134:449–452, 1980.
15. Berek JS, Hacker NF: Laparoscopy in the management of patients with ovarian carcinoma, in The Treatment of Ovarian Cancer, edited by DiSaia P, Chapter 4, W.B. Saunders, london, August, 10(2):213–222, 1983.
16. Bagley CM, Young RC, Schien PS, Chabner BA, DeVita VT: Ovarian cancer metastatic to the diaphragm frequently undiagnosed at laparotomy. A preliminary report. Am J Obstet Gynecol 116:397–404, 1973.
17. Rosenoff SH, Young RC, Anderson T, *et al.*: Peritoneoscopy: a valuable staging tool in ovarian carcinoma. Ann Int Med 83:37–41, 1975.
18. Spinelli P, Luini A, Pizzetti P, DePalo GM: Laparoscopy in staging and restaging of 95 patients with ovarian carcinoma. Tumori 62:493–502, 1976.
19. Piver MS, Lopez RG, Xynos F, *et al.*: The value of pre-therapy peritoneoscopy in localized ovarian cancer. Am J Obstet Gynecol 127:288, 1977.
20. Mangioni C, Bolis G, Molteni P, Belloni C: Indications, advantages and limitations of laparoscopy in ovarian cancer. Gyn Oncol 7:47–55, 1979.
21. Ozols RF, Fisher RI, Anderson T, Makuch R, Young RC: Peritoneoscopy in the management of ovarian cancer. Am J obstet Gynecol 140:611–619, 1981.
22. Berek JS, Griffiths CT, Leventhal JM: Laparoscopy of second-look evaluation in ovarian cancer. Obstet Gynecol 58:192–198, 1981.
23. Hassan HM: A modified instrument and method for laparoscopy. Am J Obstet Gynecol 110:886–889, 1971.
24. Griffiths CT: Surgical resection of tumor bulk in the primary treatment of ovarian carcinoma. Natl CA Institute Monograph 42:101–104, 1975.
25. Griffiths CT, Fuller AF: Intensive surgical management of advanced ovarian cancer. Surg Clinic No Amer 58:131–143, 1978.
26. Hacker NF, Berek JS, Lagasse LD, *et al.*: Primary cytoreductive surgery in ovarian cancer. Obstet Gynecol 61:413–420, 1983.
27. Schwartz PE, Smith JP: Second-look operation in ovarian cancer. Am J Obstet Gynecol 138:1124, 1980.
28. Smith JP, Delgado G, Rutledge F: Second-look operation in ovarian cancer. Cancer 38:1438, 1976.
29. Phillips BD, Buchsbaum HJ, Lifshitz S: Re-exploration after treatment for ovarian carcinoma. Gynecol Oncol 8:339, 1979.
30. Curry SL, Zembo MM, Nahhas WA, *et al.*: Second-look laparotomy for ovarian cancer. Gynecol Oncol 11:114, 1981.
31. Webb MJ, Snyder JA, Williams TJ, *et al.*: Second-look laparotomy in ovarian cancer. Gynecol Oncol 14:285, 1982.
32. Raju KS, McKinna JA, Barker GH, *et al.*: Second-look operations in the planned management of advanced ovarian carcinoma. Am J Obstet Gynecol 144:650, 1982.
33. Roberts WS, Hodel K, Rich WM, *et al.*: Second-look laparotomy in the management of gynecologic malignancy. Gynecol Oncol 13:345, 1982.
34. Gershenson DM, Copeland CJ, Wharton JT, *et al.*: Surgically-determined complete responders in advanced ovarian cancer. Proc Soc Gyn Onc 14:18, Marco Island, FL, 1983.

35. Berek JS, Hacker NF, Lagasse LD, *et al.*: Second-look laparotomy in Stage III epithelial ovarian cancer: clinical variables associated with disease status. Obstet Gynecol 64:207–212, 1984.
36. Berek JS, Hacker NF, Lagasse LD, *et al.*: Survival of patients with ovarian cancer following secondary cytoreductive surgery. Obstet Gynecol 61:189–193, 1983.
37. Young RC, Chabner BA, Hubbard SP, *et al.*: Advanced ovarian adenocarcinoma: a prospective clinical trial of melphalan (L-PAM) versus combination chemotherapy. N Engl J Med 299:1261, 1978.
38. Kapadia SB, Krause JR: Ovarian carcinoma terminating in acute nonlymphocytic leukemia following alkylating agent therapy. Cancer 41:1670–1680, 1978.
39. Reimer RA, Hoover R, Fraumeni JF, Young RC: Acute leukemia alkylating agent therapy of ovarian cancer. N Eng J Med 297:177–181, 1977.
40. Piver MS, Lele SB, Barlow JJ, Gamarra M: Second-look laparoscopy prior to proposed second-look laparotomy. Obstet Gynecol 55:571–573, 1980.
41. Lacey CG, Morrow CP, DiSaia PJ, Lucas WE: Laparoscopy in the evaluation of gynecologic cancer. Obstet Gynecol 52:708–712, 1978.
42. Smith WG, Day TG, Smith JP: The use of laparoscopy to determine the results of chemotherapy for ovarian cancer. J Rep Med 18:257–260, 1977.

6. The role of radiotherapy in cancer of the ovary

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A principal goal in treatment planning for radiation therapy, is to develop a treatment technique that will deliver an effective homogeneous dose of irradiation to the 'tumor-bearing volume', while minimizing the dose to the surrounding normal tissues. In epithelial ovarian cancer the 'tumor-bearing volume', the region of actual tumor and the tissues which are at risk, is the abdominopelvic cavity, that portion of the body cavity below the diaphragm. The peritoneal cavity is located within the abdominopelvic cavity and is divided into a greater and lesser peritoneal sac. The para-aortic and pelvic nodes are located outside the peritoneal cavity but within the abdominopelvic cavity.

The use of radiation to treat this volume of interest in epithelial ovarian cancer presents a challenge to the gynecologic and radiation oncologist.

Patient evaluation and selection

The evaluation and selection of the appropriate patient with epithelial ovarian cancer for treatment by radiation therapy is the cornerstone of successful therapy. Three criteria can be used for assessing the patient for radiation therapy are: the extent of the disease process, i.e., stage; the histologic type and grade, and initial tumor volume and/or residual tumor volume.

If the extent of the disease process demonstrates dissemination outside the abdominopelvic cavity, irradiation as primary therapy is deemed inappropriate. This may be documented by inguinal and/or supraclavicular node involvement, positive cytology from a pleural effusion, or metastatic chest lesions. Patients who have documented ovarian cancer in the parenchyma of the liver are not primary radiation therapy candidates. In summary, any ovarian cancer patient who demonstrates disease outside the abdominopel-

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vic cavity or who has parenchymal liver involvement, is not a candidate for primary radiation therapy. These patients are better served by chemotherapy as radiation can only limit the dose of chemotherapy and delay treatment because of prolonged marrow suppression.

Approximately 70 per cent of patients with epithelial ovarian cancer at initial presentation will have widespread intraperitoneal and/or retroperitoneal involvement [1]. The intraperitoneal involvement extends from the thoraco-abdominal diaphragm to the pelvic diaphragm. All intraperitoneal organs, abdominal recesses and retroperitoneal nodes are at risk for tumor involvement. In these patient, it is size and location of the residual tumor that determines who is a candidate for radiation therapy. Patients with tumor located on the peritoneum of the kidney are not suitable radiation candidates, because of the limited radiation tolerance of the kidney of 2000–2500 rad [2]. Patients with extensive implants on the liver also must be evaluated carefully because of the radiation tolerance of the liver of 2500 to 3000 rad [2]. Tumor located in the lesser sac, and involvement of the retroperitoneal space in the form of para-aortic and/or pelvic nodes are difficult target volumes to deliver therapeutic radiation and still maintain an acceptable complication rate. This is based on a radiation dose of 5000 rad that is required to obtain control, for residual masses 1.0 centimeters or less in diameter, while for nodules greater than 1.0–2.0 centimeters, doses greater than 5000 rad may be necessary [2–4]. These doses may exceed the tolerance of the overlying stomach and small intestine which has a tolerance of between 4500 to 5500 rad. The patient, with what appears to be localized disease, but on a meticulous surgical staging is found to have disseminated disease with no visible residual disease is a candidate for radiation therapy. As occult peritoneal metastases may be controlled with doses as low as 2500 rad [4]. Those patients who are not appropriately and accurately staged so as to have the 'tumor-bearing volume' defined are therapeutic dilemmas in deciding what is the appropriate therapy. Suitable radiation candidates are those that had a meticulous intraoperative evaluation and have minimal residual disease, less than 1 centimeter, at the time of surgery.

The histologic type and grade of the epithelial ovarian cancer is an important prognostic and selection variable. The biologic aggressiveness of all epithelial ovarian cancer appears equal when compared by grade and stage. It has also been shown that all epithelial ovarian cancers are equally radioresponseve to radiation therapy. Thus, there is no need to select individuals based on histologic types. The radioresponsiveness of ovarian cancer, the rate at which clinical manifestations of radiation-induced biologic change takes place is not rapid. The radiosensitivity of ovarian cancer is based on the ability of radiation to produce cell kill or destrution of reproductive capacity. Evidence now exits that indicates that all epithelial tumors have the same radiosensitivity provided the volume of cancer and the tumor bed

are similar [5]. The radiocurability of epithelial ovarian cancer is dependent, not on histologic type, but on the prognostic factors of stage, residual tumor and tumor location. The grade of the epithelial cancer is an important consideration regarding therapeutic alternatives. Radiation therapy should be reserved for those patients with a 'true invasive cancer'. Patients with a tumor classified as being of borderline malignancy, grade 0, or of low malignant potential should not be considered candidates for radiation therapy. This is based on their histologic appearance of having less than one mitoses per high power field and an absence of stromal invasion. The survival data of these tumors is superior to that of invasive carcinomas. Santesson and Kottmeier reported a ten-year survival for borderline serous tumors of the 76 per cent with a corresponding figure of 13 per cent for serous cystadenocarcinomas [6]. The histologic type and grade of the epithelial ovarian cancer should not eliminate cases for consideration.

The 'residual tumor volume', that amount of tumor remaining after attempts at surgical reduction of the presenting tumor mass, is an important determinant for the effectiveness of radiation therapy. This surgery has been termed cytoreductive surgery, tumor debulking, or maximum surgical effort. Tumor masses larger than 2 centimeters have been shown to be a poor prognostic indicator for radiation or chemotherapy [7]. As stated previously, tumor nodules greater than 1.0–2.0 centimeter may require radiation doses greater than 5000 rad to obtain local control and may, depending on the tumor location exceed the tolerance of the normal adjacent tissues. The available data appears to show that a reduction in 'residual tumor volume' to less than or equal to 2 centimeters has a similar prognosis to patients who present with this optimal tumor volume [8]. The difference between the two groups is the degree of surgery required to achieve this 'residual tumor volume'. The more aggressive the surgical approach, the greater the possibility of radiation associated complications due to adhesions and compromised vascular supply.

The patient who has ascites is a problem in treatment planning due to the changing abdominal configuration and the possibility for continuous abdominal seeding from free-floating tumor cells.

The careful evaluation and selection of the appropriate patient with epithelial ovarian cancer for primary radiation therapy cannot be over-emphasized.

Available modalities

The modalities available in the radiation management of ovarian cancer are those of teletherapy and radioisotopes.

Teletherapy

The first account of radiation therapy in the treatment of carcinoma of the ovary was first made by Eymer in 1912 [9]. The development of megavoltage radiotherapy equipment with energies in the range of 1 MeV or higher has allowed the delivery of curative doses of radiation to tumors without prohibitive toxicity to normal tissues. Megavoltage radiation beams are more penetrating than the lower energy beams that they replaced, are not preferentially absorbed in bone and minimize the radiation to the skin. Megavoltage beams can be provided by ^{60}Co units, betatrons and linear accelerators. The energy of the cobalt units is lowest and hence, their beams are less penetrating, and, the edges of the beam are not as sharply defined as those produced by linear accelerators producing a penumbra or radiation dose at the edge of the field. Radiation therapy should thus be delivered by megavoltage equipment, such as ^{60}Co units, 4- to 6- MeV linear accelerators, or 25- to 35- MeV linear accelerators.

Treatment of the 'tumor-bearing volume', defined as the abdominopelvic cavity, can be approached in a number of ways. Ideally, the irradiation to the whole abdomen should be given in one undivided volume with a homogeneous dose. The upper border of this target volume is the thoraco-abdominal diaphragm. Verification films should be taken, in expiration, to ensure coverage of the diaphragm by at least one centimeter. The large volume that would be irradiated at one time results in poor patient tolerance. Toxicity is primarily intractable nausea and vomiting which often results in unacceptable treatment delays. The maximum dose that can be delivered, is approximately 3000 rad in five to six weeks, with proper shielding of the kidneys and liver [10]. There have been attempts to modify this basic approach to improve tolerance, by using three or even four fields to encompass this same target volume [10]. The results are the same as the tissue volume irradiated at one time is the same.

The technique used at The Johns Hopkins Hospital is to divide the abdominopelvic region into two fields. Irradiation is from a Cobalt 60 source with 200 rad daily fractions to the pelvis and 150 daily fractions to the upper abdomen with posterior kidney blocks (6 HVL). The pelvic field is from the ischial tuberosities to a point above the iliac crests extending to the lateral pelvic wall with a two centimeter margin. The iliac crests are excluded by appropriate blocks. The upper abdominal field extends from above the iliac crest to a region two centimeters above the dome of the diaphragm and laterally at least to the peritoneal reflection. The abdominal and pelvic fields are separated by a calculated gap and meet at the mid plane with a plus or minus 5 per cent homogeneity. Each day, pelvic irradiation is followed two hours later by irradiation of the upper abdomen. The patients are treated five times a week to a total dose of 4000 rad to the

pelvis and 3000 rad to the upper abdomen. There has been minimal toxicity and no acute morbidity associated with this technique.

The ^{60}Co moving strip technique was developed to allow delivery of a biologically more effective dose that would be better tolerated [10, 11]. Lines, 2,5 centimeters apart, are marked on the front and back of the patient. For two days, the lowest strip is treated from the front and the identical strip is treated from the back. A 2.5 centimeter strip is then added and the two strips are treated for two days. Subsequently, each added strip is treated for two days before an additional strip is added. When 10 centimeters, 4 strips, are treated for two days, one strip is added cephaled, and the most caudad strip is deleted. This is repeated every other day until the uppermost strip is reached. When the strip irradiation is completed, whole pelvis radiation can then be added. A total dose of between 2000 rad and 2800 rad can be delivered to the midplane by this technique. Shielding of liver and kidney is dependent on dose. A modification of this technique is required when using a 4 MeV photon beam of a linear accelerator because of the sharpness of the beam [12]. A dose of 250 rads is given each day alternating between anterior and posterior strips. To avoid cold spots the anterior and posterior strips are staggered so that the posterior surface line projects to the center of the anterior opposed strip. One strip is added superiorly until four strips are treated and then the most inferior strip is omitted. The objections to these moving strip techniques are that they are difficult to do technically and to accurately reproduce the treatment fields. It requires from 40 to 60 days to accomplish the moving strip portion and during this period only 10 centimeters are treated at a time, with the possibility of tumor reseeding from above and below the area being treated.

Variations on the moving strip theme include starting the moving strip at the dome of the liver, eliminating shielding to the liver by reducing the total dose to 2250 rad delivered by the moving strip technique. Starting the strips cephalad permits treating the whole pelvis simultaneously with the upper abdominal strip.

Evaluation of the moving strip technique versus large opposed field irradiation has failed to demonstrate any difference between the two techniques [13, 14].

Brady has proposed total abdominal irradiation to dose levels that would be appropriate for the disease being treated, as well as volumes that would include the entire peritoneal surfaces and pelvis, carried out by the field-within-a-field technique [15]. An example of this technique is used at Stanford [16]. The true pelvis is initially irradiated, from L-4 to the obturator foramen, to 900 rad in one week at 180 rad per fraction. The field is then opened to include the entire abdominal peritoneum, extending from the obturator foramen to one centimeter above the diaphragms, and treated to a total dose of 3000 rads at 150 rad per day. Kidney blocks are placed at 1000

rads and liver blocks at 1500 rads. A break of one week is given bevore 1200 rads of radiation is given to a 'T-shaped' field covering the para-aortic nodes and medial half of each diaphragm.

Arcangeli *et al.* have recently developed a chessboard-like technique of abdominal and pelvic irradiation that takes advantages of the reproductive patterns of the intestinal cells and the slowly or nonproliferative cancer or connective tissue cells [17].

Irradiation of the whole abdomen presents a situation of compromise, by either limiting the 'targer volume', by introducing shielding, reducing the field size, or using a dose of irradiation that is within the tolerance of the liver, kidney and bowel. There appears to be no improvement in tumor control if the dose is increased at the expense of a reduced volume. In summary, teletherapy of ovarian cancer, still remains a challenge due to the large 'tumor-bearing volume' that must be treated within the confines of the toxicity of the abdominal contents.

Radioisotopes

Another modality of therapy in ovarian cancer is intraperitoneal radioisotopes. The first reported use of intraperitoneal radioisotopes was by Muller in 1945 [18]. He instilled radioactive zinc, ^{63}Zn, into the peritoneal cavity as a palliative treatment for control of malignant ascites secondary to ovarian cancer. Hahn prepared the first colloidal suspension of radiogold, and Muller reported on its use [19, 20]. Muller presented his results with radioactive gold in patients with ovarian cancer treated for cure [21]. The first reports of the use of intraperitoneal radioactive colloid in the United States were by Keettel and Elkins and Card, Cole and Henschke [22, 23].

The use of intraperitoneal ^{198}Au resulted in a significant incidence of major complications, and associated deaths [24–28]. In reviewing seven series with over 800 patients where a dose of 150 to 200 mCi of radioactive gold (^{198}Au) was used, an unacceptable complication rate is reported. Complications resulted in the death of four patients. The explanation for complications associated with ^{198}Au can be attributed to its gamma component, the short physical half-life (2.69 days), an excessive dose, or a combination of these factors.

The beta and gamma components in the disintegration of ^{198}Au are represented diagrammatically in Figure 1 [29].

In the disintegration of one ^{198}Au atom three beta particles are emmitted, $beta_1$, $beta_2$ and $beta_3$ with a distribution of 1.3 per cent, 98.6 per cent and 0.02 per cent respectively. In more than 99 per cent of the disintegrations, each beta particle has as an associated gamma ray. The mean energies of the beta particles and gamma rays are shown in Table 1 [29].

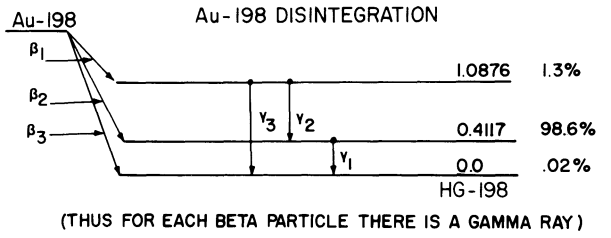

Figure 1. The disintegration of radioactive colloidal gold (^{198}Au) by the emission of 3 beta particles and 3 gamma rays, to a stable state of mercury.

Table 1. Mean energies in the disintegration of Au-198

Mean Kev energies	Beta	Mean Kev energies	Gamma
$Beta_1$	81.1	$Gamma_1$	411.7
$Beta_2$	316.3	$Gamma_2$	675.8
$Beta_3$	464.8	$Gamma_3$	1087.6

Beta particles in the disintegration ^{198}Au are negatively charged particles (electrons) with a rest mass of about 0.000548 atomic mass units [30]. The depth of penetration of a beta particle in tissue is dependent upon its energy. The $beta_2$ particle of ^{198}Au has a mean energy of 411 keV. Gamma radiation with no rest mass or charge may penetrate deeply into matter or tissue. ^{198}Au, then, has a short-range radiation component, beta radiation, and deeply penetrating gamma component [31]. The gamma radiation presents a hazard to the patient and medical personnel caring for the patient.

The short half-life of ^{198}Au (2.69 days) is responsible for rapid dose deposition. This may, in part, be responsible for the associated complications. Muller empirically determined a dose of 100 to 200 mCi for the intraperitoneal administration of radiogold [21].

Muller attempted to determine the dose absorbed in intra-abdominal tissue due to the beta and gamma radiation from 150 mCi of ^{198}Au. The absorbed dosages were obtained from neutron activation analysis of tissue samples from patients who died one to two weeks after injections of ^{198}Au. His values were 4000 rad to the peritoneum, 6000 rad to the omentum, 7000 rad to the retroperitoneal lymph nodes, 250 rad to the spleen, 170 rad to the liver and 30 rad to each kidney. He also calculated that '750 rad of diffuse penetrating gamma radiation must be added' [21]. The dosages are very difficult to interpret because of the following unknowns: the number of patients studied; the methodology utilized; the clinical status of the patients prior to injection of ^{198}Au; the cause of death; the distribution of ^{198}Au at

the time of instillation; the number and range of dose measurements; the permanence of the fixation of colloidal gold to tissue; and the method used for determination of the gamma radiation dose. Thus, these values, so frequently quoted in the literature for this dose of ^{198}Au, should be used with the utmost caution.

Since 1955, ^{32}P has been the agent of choice for intraperitoneal instillation for both treatment of ovarian cancer and palliation of malignant ascites [23]. Jones, Wrobel and Lyons developed a chemically inert colloidal form of ^{32}P in 1944 [32]. The advantages which led to the choice of ^{32}P over ^{198}Au are: higher beta energy and, therefore, greater tissue penetration; a longer half-life; and an absence of gamma radiation [23, 26, 33–37]. The experience with intra-abdominal ^{32}P has demonstrated minimal complications.

The dose of intraperitoneal ^{32}P was estimated from the empirical dose of ^{198}Au. A dose of 10–15 mCi of ^{32}P was thought to be equivalent to 100–150 mCi of ^{198}Au because of a longer half-life of 14.3 days and more energetic beta particles of 0.69 MeV of ^{32}P [33, 38]. Silver, Blahd and Van den Brenk *et al.* have each made statements regarding the dose relationship between ^{32}P and ^{198}Au: (1) 'The energy delivered by 1 mCi of the chronic phosphate in 1 ml of a solution is about 885,000 rep, or more than ten times that of a solution of colloidal gold of the same activity.' (2) 'In comparison to ^{198}Au, chromic phosphate ^{32}P has a greater penetration and a greater destructive action per disintegration. One microcurie per gram of chromic phosphate ^{32}P will deliver 885 rad in contrast to one microcurie of gold ^{198}Au, which will deliver 76 rad. Furthermore, ^{32}P has a longer half-life than ^{198}Au, and its beta radiation is more energetic and, therefore, more penetrating [1]. Consideration of these physical properties means that provided the material is not lost from the cavity 10 mCi $Cr^{32}PO_4$ will deliver approximately the same amount of radiation to the serous wall as 100 mCi of ^{198}Au [38–40]. In arriving at these conclusions, it was assumed that ^{32}P, remains confined to the peritoneal cavity and plates out uniformly over the serous surfaces. Thus, the clinical value of a dose of ^{32}P, based on these assumptions, and calculated from an initial empirical dose of ^{198}Au, must be viewed with scepticism and concern.

Clinical experience would also indicate that the dose delivered by ^{32}P is less than an equivalent dose of ^{198}Au. Hester and White used multiple infusions of 7 mCi of ^{32}P; 32 patients received more than one intraperitoneal instillation [36]. The largest group, 18 patients, recorded four injections for a total of 28 mCi and one patient received six injections for a total of 42 mCi. Associated complications were minimal [36]. Tissue radiation dose delivered by this type of dose fractionation is unknown. Our experience with the single intraperitoneal administration of 30 mCi of ^{32}P without complications, would suggest that 100 mCi of ^{198}Au delivers a far greater

tissue dose than 10 mCi of ^{32}P. The optimal intraperitoneal dose and effect of dose fractionation on tissue are unknown.

The information currently available for actual radiation doses delivered by ^{32}P after intraperitoneal administration is limited, as the dose absorbed *in vivo* is dependent not only upon the physical properties of the radionuclide, but also on how rapidly the radionuclide is distributed by biological processes, and the uniformity of distribution [31]. The factor of elimination of ^{32}P was studied by Root *et al.*, showing that, over a period of eleven days, less than 1 per cent of the administered dose was found in the blood and approximately 5 per cent of the administered dose was excreted in the urine [41]. The patients studied had recurrent pleural effusions and ascites so that the peritoneal dynamics cannot be considered normal. Therefore, the Root data are difficult to interpret and extrapolate to patients without ascites [41]. The precise distribution of radiophosphorus in patients is not known. Consequently, calculations of radiation dosages delivered by ^{32}P which are based on the assumptions of uniform distribution and static state cannot be applied to the *in vivo* situation.

The current commercially available chromic phosphate is a colloidal suspension of chromic phosphate molecules in a solution of 30 per cent glucose and 2 per cent benzyl alcohol. Table 2 summarizes the physical properties of chromic phosphate. Though not a true colloid, ^{32}P exhibits many of those properties and can be referred to as a colloidal suspension have a surface area much greater than the volume and, thus, the particles exhibit adsorption to other molecules. In the colloidal phosphate suspension, there is adsorption to the glucose.

In order to evaluate the percentage of bound to chromate and to ensure minimal quantities of free ^{32}P, thin layer chromatography is done. Usually 98 per cent is bound and 2 per cent is free ^{32}P.

The radiation emitted by ^{32}P is in the form of electrons. The nucleus acts as a negatron emitter. In this process, a neutron within the nucleus is converted to a proton, an electron and a neutrino [42]. In this transformation,

Table 2. Physical properties (32p)

Chemistry	Colloidal suspension
pH	3.5
Particle size	.5–1.5 microns
Physical half life	14.3 days
Average Beta energy	.69 Mev
Maximum Beta energy	1.7 Mev
Average tissue range	1.4 to 3 mm
Maximum tissue range	8 mm
Color	Blue-Green

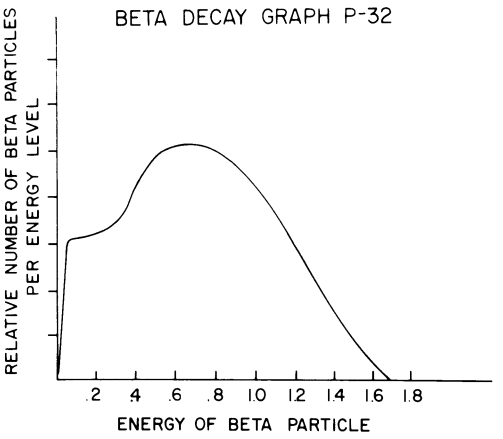

Figure 2. The distribution of energies of the beta particles liberated in the disintegration of ^{32}P, Johns and Cunningham [42].

the mass number of ^{32}P remains the same, 32, and the charge number increases to 16 because of the addition of one proton to the nucleus. The final product is stable sulphur $^{32}S_{16}$ [42]. The maximum energy of the emitted beta particle is about 1.7 MeV; the average maximum energy is approximately 0.69 MeV. The distribution of the energy liberated in this reaction is shown in Figure 2 [29, 42].

The half-life of $^{32}P_{15}$ is 14.3 days. Thus, in 14.3 days, half the atoms will disintegrate and in the next half-life, one half the remaining atoms will decay; this is an exponential form of decay. The half-life decay of ^{32}P is depicted in Figure 3 [42].

Dose rate is an important parameter in determining whether the tumor will respond. The dose rate of ^{32}P being low requires a high specific activity to achieve dose rates that are effective. It is calculated that it would require five to ten times the normally administered activity to achieve these dose rates [43].

The method of instillation of ^{32}P is determined by whether an intraperitoneal catheter or catheters are placed at the time of surgery or in the post-operative period.

A catheter or catheters placed at the time of surgery can be nylon or Silastic tubes sealed at one end with multiple perforations. One catheter is placed over the liver and the other is placed in the pelvis. The catheters are brought through the abdominal wall and fixed to the abdomen with two circumferential purse string sutures. The catheters are kept patent by instillation of a heparin solution. Catheter placement at the time of surgery is preferred as it avoids further invasive procedures.

Catheter placement in the post-operative period may be accomplished by laparoscopic visualization with placement of the catheters over the dia-

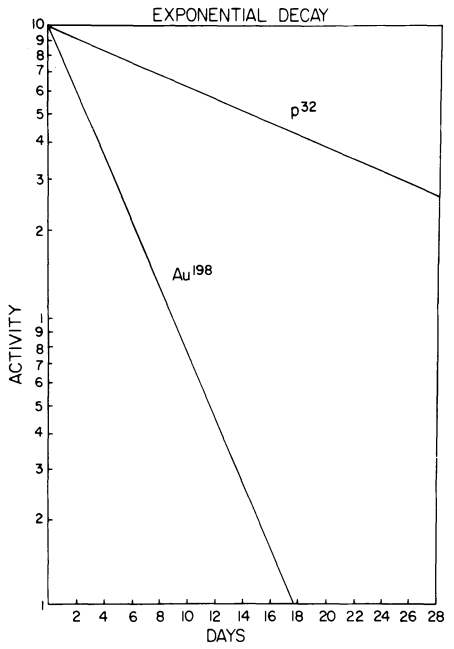

Figure 3. Exponential decay of both ^{32}P and ^{198}Au with the rapid dose deposition of ^{198}Au as opposed to ^{32}P, Johns and Cunningham [42].

phragm and in the pelvis. Laparoscopic placement permits assessment of the abdomen and direct placement of the catheter.

Horowitz described a technique of post-operative placement by abdominal insufflation with carbon dioxide gas and then placement of the catheter into the peritoneal cavity [44]. This catheter may be either a Longdwel® catheter or angiocath. Finally, the 'blind placement' of either a Longdwel® or angiocath-type catheter in the right quadrant without gas insufflation or laparoscopic visualization may be done. All catheters placed for ^{32}P should be sutured to the skin in order to keep their position within the abdominal cavity.

Prior to instillation of ^{32}P, the position of the catheter and distribution of injection material must be determined (Figure 4). This is most easily done by the injection of 30 cm^3 of Renografin-60® via the catheter(s), with determination of free flow within the peritoneal cavity as evidenced by dispersion of the radio-opaque material within the abdominal cavity on diagnostic quality radiological films. If the Renografin-60® is loculated, confined to the area of injection, there should be reinsertion of the catheter into a different location with reinjection of Renografin-60® to ascertain uniform distribution.

It has also been suggested that an abdominal radionuclide scan should be carried out prior to injection of the radioactive phosphorus by injecting

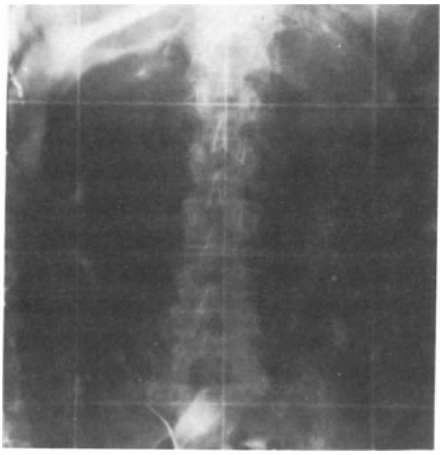

Figure 4. Free flow of Renografin-60® after intraperitoneal administration.

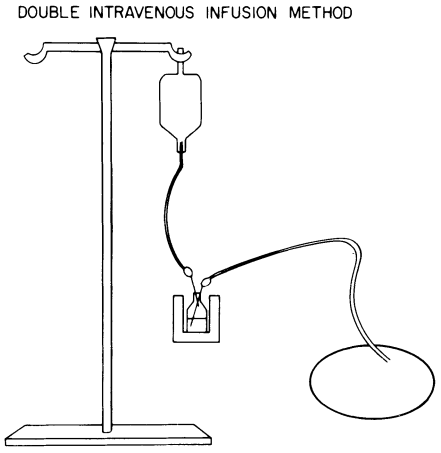

Figure 5. Technique of double intravenous infusion of ^{32}P from ^{32}P container to patient.

2–3 mCi of technetium sulphur colloid via the peritoneal catheter, followed by total abdominal scanning to determine the potential distribution of the colloid [45–47].

The instillation of ^{32}P into the abdominal cavity is accomplished by means of a double intravenous infusion set-up. An intravenous infusion of isotonic saline is placed into the container of ^{32}P and then tubing placed from the container to the patient catheter (Figure 5).

The instillation of ^{32}P should be scheduled after full recovery from the abdominal operation. We elect to instill the ^{32}P immediately prior to the patient's discharge from the hospital. No special shielding requirements or patient precautions are necessary because of the pure beta radiation of ^{32}P.

The physician should wear gloves and all materials and tubing used in the process of instillation should be given to the Radiation Safety Officer.

The precise distribution of intraperitoneally administered ^{32}P is not known. There appears to be both an abdominal distribution and systemic distribution of ^{32}P. A portion of the administered ^{32}P is either adsorbed to the peritoneal surface, absorbed by the macrophages [39, 48]. The remainder of the ^{32}P is carried by the abdominal current to the right hemidiaphragm where it passes through the diaphragmatic lymphatics and enters the mediastinal lymphatics. It then passes to the right subclavian vein via the right throacic trunk and enters the general circulation. The ^{32}P is rapidly cleared by the liver and to a lesser extent deposited in other tissues including lungs, kidneys, spleen and bone marrow [49, 50].

Vider, Deland and Maruyama reviewed their experience with scintillation camera imaging of technetium pertechnetate, instilled simultaneously with the ^{32}P as an indirect way of determining ^{32}P [51]. In four cases studied, all showed an uneven distribution. This was interpreted as non-uniformity of dispersion of ^{32}P within the abdomen.

Knowledge of the distribution of ^{32}P within the abdominopelvic cavity is essential in determining the therapeutic efficacy of ^{32}P. Investigators have attempted to define both distribution and dose distribution.

The scanning of the actual ^{32}P distribution by bremsstrahlung has been suggested [52]. Bremsstrahlung or 'braking' radiation, the result of beta emissions interacting with atomic nuclei with the production of a continuous spectrum of x-ray energies, can be measured by scanners. Kaplan *et al.* used an Anger camera for sequential pelvic, abdominal and thoracic scintigraphic recording of bremsstrahlung radiation from ^{32}P over a three-week period [53]. They determined that there was rapid and persistent focal aggregation of the radiocolloid with no change in pelvic or peritoneal radionuclide distribution patterns. Dispersion of the ^{32}P was rapid an related to peritoneal dynamics.

Investigators have attempted to answer the essential question of ^{32}P distribution and dose distribution in animal models. In our rabbit model the ^{32}P was not uniformly distributed over the peritoneal surfaces and many areas were minimally radiated [54]. Increasing the injection volume had no significant effect on distribution or tissue doses. Currie *et al.* demonstrated, in dogs, that pelvic and para-aortic lymph nodes receive minimal dosages of radiotion [55]. Significant activity was demonstrated in the thoracic lymph nodes. Their work with thermoluminescent dosimeters and Geiger-Muller tissue counting indicate that ^{32}P 'probably' delivers therapeutic dosages. Tewfik *et al.* also used dogs and showed a lack of uniform distribution of ^{32}P [56]. All three groups demonstrated significant activity in the diaphragm.

To obtain uniform distribution throughout the abdomen, many authors

have suggested position changes after introduction of the therapeutic radioactive colloid. Myers demonstrated that one could accomplish this Trendelenburg position [57]. Buchsbaum, frame to accomplish positional change and distribution [58]. Rosenshein *et al.* in monkey experiments on intra-abdominal distribution suggested that the patient change position and that the initial vehicle used for instillation be of sufficient quantity to mildly distend the abdomen in order to maximize distribution [59]. Currie *et al.* have shown that pre-mixing the ^{32}P in a large volume may assist in the distribution [55].

In summary, the precise distribution and dose of intraperitoneal ^{32}P to various areas in the abdomen are unknown.

Combined therapy

Several investigators have combined intraperitoneal radiocolloids ^{198}Au or ^{32}P and external beam radiation [21, 28, 60, 61]. The Johns Hopkins Hospital protocol for the radiation management of the suitable candidate with ovarian cancer combines the instillation of 15 millicuries of ^{32}P with the previously mentioned external beam therapy protocol. The external radiation is started two weeks after the instillation of 15 millicuries of ^{32}P which is instilled two weeks after laparotomy.

Results

This section will present the results of treatment, by radiation therapy, according to the FIGO staging system. The basis of this presentation, along with future work, is dependent upon the accuracy of the definition of the 'tumor-bearing volume'.

Studies, present and future, that accept into their study cases that are not aggressively staged all to the confusion that already exists in the literature regarding the proper management of ovarian cancer. Brady has stated this in another way, 'In large measure the multiple studies reviewed fail to indicate the benefit of radiotherapy in ovarian cancer, but in every instance the treatment program was inadequate to provide for the extent of disease as we know it to be today' [15].

The results of treatment of ovarian cancer will be presented under two divisions – limited (Stage I) versus disseminated (Stage II, III). Limited ovarian cancer is defined as disease confined to the ovary, with or without rupture, and with or without positive peritoneal cytology, i.e. Stage I ovarian cancer. The patients with FIGO Stage II and III, are classified as disseminated cancer.

Stage I – ovarian cancer

No patient should be classified as having Stage I cancer unless a meticulous staging has been performed. In a group of well-staged epithelial ovarian patients, the questions are: is post-operative therapy required for all patients; what form should post-therapy take when indicated; and what benefits do post-operative therapy convey?

The answer to the first question is that it is not necessary that all patients receive post-operative adjuvant therapy. Careful patient selection appears to eliminate a proportion of the well-staged Stage I ovarian cancers. The patients that do not appear to require therapy are those with a unilateral lesion, that is well differentiated, without rupture or adhesions, and with negative peritoneal washings.

What form the post-operative therapy should take can be determined from sites of recurrence in treated patients. It appears that the sites of recurrence extend throughout the peritoneal cavity, thus, when treatment is indicated, it should be directed at the whole peritoneal cavity. The problem facing the physician is that Stage I is an uncommon presentation of ovarian cancer, especially when a thorough intraoperative staging exploration is also performed. In this group of patients, there is reluctance to recommend use of very toxic therapy since certain patients can be cured by surgery alone, and simple treatment methods have failed to show a survival benefit.

Dembo and Bush presented a randomized study of 54 Stage Ia ovarian cancers [61, 62]. Twenty-seven patients were treated by 4500 rads, in 20 fractions, to the pelvis and an equal number were only observed. There was no significant difference between the nine relapses in the radiation group and 14 in the control group. The pattern of recurrences were such as to indicate that the entire peritoneal cavity was at risk. Therefore radiation to the pelvis was thought to be inadequate. The GOG study randomized Stage I patients between observation, pelvic radiation and melphalan [64]. No

Table 3. Survival with surgery and adjuvant Au-198 Stage I ovarian cancer

Author	Dose/mCi	Stage	No. pts.	5-Year survival
Buchsbaum [69]	100	Ia	56	94.3%
Moore [70]	100 to 200	Ia		90.5%
Decker [27]	140	I	56	73.2%
Perez [71]	150	I	8	50.0%
Keettel [22]	150	I	37	86.0%
Keettel [25]	150	Ic	44	73.0%
Rose [72]	100 to 200	Ic	24	75.0%
Fletcher [73]	N.S.	I	18	78.0%

Table 4. Survival with surgery and adjuvant ^{32}P Stage I ovarian cancer

Author	Dose/mCi	Stage	No. pts.	Survival
Hester [36]	7 to 42	I	9	89.0%[1]
Clark [26]	15	I	28	92.5%[2]
Piver [37]	10	Ia	18	94.0%[3]
Julian [13]	15	Ia_1	6	83.0%[4]
		Ib_2	3	100.0%
		Ic	3	67.0%
Piver [14]	15	Ia	12	100.0%[5]
		Ib	4	75.0%
		Ic	4	100.0%

[1] Varying time.
[2] Year.
[3] 3 to 13 years vs surgery alone 22.2%.
[4] 3 year.
[5] 1.5 to 6 years followup.

statistical difference was noted between the observation group and the pelvic radiation and melphalan group. Of the 14 tumor relapses, three were confined to the pelvis thus most of the relapses were distributed throughout the peritoneal cavity. The study results have been questioned because 49 per cent entered on the study were removed from the analysis, thus bias between groups could have occurred. In both studies, cases were accepted that were not totally staged.

The M.D. Anderson compared melphalan administered in 12 cycles to whole abdominopelvic radiation, 2600 to 2800 rad by an upward moving strip irradiation plus a pelvic boost of 2000 rad [65, 66]. The preliminary projected five-year survival for Stage I was 100 per cent for the radiation group and 86 per cent for melphalan. No significant survival difference is noted at six years between the two groups.

Radiocolloids have also been used in the treatment of Stage I ovarian cancer. A series of patients treated with surgery and intraperitoneal radiogold had a higher five-year survival than the 60 to 70% expected for surgery alone except for one group of eight patients [67, 68] (Table 3).

The results of treatment with intraperitoneal ^{32}P and surgery in Stage I ovarian cancer are reviewed in Table 4. Clark and Piver, Barlow and Lele indicate survival data in the 90 per cent range [16, 37]. In 12 patients treated at The Johns Hopkins Hospital, there was an 85 per cent three-year survival [74].

In Stage I ovarian cancer, there appears to be circumstantial information that intraperitoneal ^{32}P can improve survival over surgery alone.

The effectiveness of intraperitoneal therapy should be analysed by stage, histological type and grade of tumor. In evaluating ^{198}Au therapy, Decker's

series had 72 per cent of their Stage I cancer being either a grade 1 or 2 [27]. It would appear that part of the effectiveness of ^{198}Au therapy in Stage I disease may, in part, be related to the well differentiated nature of the tumors treated. Regarding histological types, mucinous tumors had 47 per cent five-year survival, endometroid 62.5 per cent, serous 57 per cent, and solid 50 per cent; however, these results included all grades and stages. There is insufficient available data to determine the relative effectiveness of radiocolloids by tumor type. Results correlating histological type and grade in Stage I disease treated by intraperitoneal ^{32}P alone are scant. The data generated by our institution were that, of the nine Stage I epithelial lesions treated, three were borderline and six were well differentiated lesions. Again, the effectiveness of therapy may have been partially influenced by the well differentiated nature of the tumor.

In a recently published article Piver reported the results of 20 patients treated with ^{32}P with a 95 per cent survival [75]. This report fails to answer the crucial question whether the excellent results are due to the patient population or to the treatment. No patient had had a thorough and complete staging, but 12 were called 'Stage Ia' and 11 were grade 1 lesions. The patient population thus looked to be a low risk one. The one death occurred in a Stage I grade 2 lesion.

The adjuvant treatment of the well staged, Stage I ovarian cancer remains to have defined which patients require treatment and what is the optimal treatment.

Disseminated ovarian cancer

All patients which after aggressive surgical staging cannot be assigned to a Stage I ovarian cancer should be classified as having disseminated disease. Patients with disseminated epithelial ovarian cancer confined to the abdominopelvic cavity, have had a variety of radiation therapy techniques employed. Interpretation of the older literature is difficult because radiation techniques were variable in energy, total dose and field size. The studies were usually not randomized and equivalence of patients treated by the modalities being compared cannot be evaluated with respect to prognostic variables.

The Princess Margaret Hospital has presented a large experience with ovarian cancer [62, 63, 76, 77]. Their conclusions were that cures are rarely obtained when there was 'large residual disease', and as abdominopelvic irradiation is associated with significant acute symptoms its use in this group of patients is not indicated. The benefit of abdominopelvic irradiation is greatest when there is no visible disease or small residual disease. 'Large residual disease' is not defined. 'Small residium patients' is not pre-

cisely defined other than it refers to macroscopic residual disease present after total abdominal hysterectomy and bilateral salpingo-oophorectomy. It is stated that 'small residuum' is not synonymous with a diameter of less than 2 centimeters. In the 'small residuum patient', Stage III, abdominopelvic radiation obtained better results than pelvic irradiation, 4500 rad midplane, in 20 fractions, followed by chlorambucil 6 mgm per day for two years. The abdominopelvic radiation consisted of pelvic irradiation of 2250 rad followed by a moving strip of the abdomen of 2250 rad. Limitations exist over interpreting the studies from the Princess Margaret Hospital because meticulous staging and aggressive attempts at tumor debulking appear not to have occurred.

A study by the GOG group of Stage III ovarian cancer stratified by extent of residual disease and randomized to either melphalan (18 cycles), whole abdominal radiation 2000 to 2500 rad plus pelvic boost to less than or equal to 3000 rad, radiation followed by melphalan, or melphalan followed by radiation [78]. There was no significant differences in survival among four treatment groups, but 61 per cent of the patients were excluded from the final analysis and liver and kidney shielding were allowed during irradiation.

In the previous section on Stage I epithelial ovarian cancer, the M.D. Anderson study of melphalan compared to the moving strip irradiation technique was cited [65]. This also included Stage III patients who had no gross residual disease or had residual disease less than 2 centimeters but not in areas that would be shielded from radiation [77]. There was no significant difference between the five-year survival of 40 per cent for radiation and 53 per cent for melphalan. As stratification for all prognostic variables was not incorporated the outcome of the study is questioned [77].

In conclusion the dose limitations of the liver, kidneys and a large volume of small bowel compromise control of macroscopic disease in the upper abdomen. Residual pelvic disease less than 2 centimeters in diameter can be controlled by pelvic irradiation in 90 per cent of cases by 5000 rad. The effect of control of pelvic disease is obviated if upper abdominal disease cannot be controlled [79]. It would appear that control of disseminated ovarian cancer by radiation is effective in patients with optimal residual disease but control may be dependent on grade of tumor [80].

The decision between the therapeutic alternatives of multiagent chemotherapy and radiation should be restricted to the group of patients with minimal or no residual disease. In this patient population data is not available concerning the superiority of one modality over the other.

Radiation as second line therapy

The recent advances made by cytoreductive surgery and intensive combi-

nation chemotherapy have brought a higher complete clinical response rate. A subset of patients who are considered to have a complete clinical response and have a 'second look' procedure which demonstrates residual tumor present a therapeutic enigma. If the residual tumor is minimal, defined as no visible residual tumor or disease less than 2 centimeters, the patients are candidates for radiation therapy. The problems are that tolerance to radiation is poor due to severe myelosuppression following intensive chemotherapy and the enteric morbidity associated with patients who have undergone at least two laparotomies. The result at The Johns Hopkins Hospital with the technique of intraperitoneal ^{32}P and split whole abdomen radiation demonstrated poor tolerance to therapy and failed to demonstrate tumor control in our heavily pretreated chemotherapy group of patients. Hainsworth reported that 14 of 17 patients relapsed at a median of eight months after radiotherapy in this group of patients [81]. The planned radiotherapy was 3000 rad to the whole abdomen via anterior and posterior opposed portals, which included the entire abdomen and pelvic peritoneum and extended from 1 centimeter above the domes of the diaphragm to the bottom of the obturator foramen.

Summary

The use of radiotherapy in epithelial ovarian cancer has to be restricted because of the large volume at risk in this disease, and limitation of dose imposed by tissue tolerance in this volume. Judicious patient selection can define a patient population with the potential to benefit from radiotherapy.

References

1. Aure JC, Hoeg K, Kolstad P: Clinical and histologic studies of ovarian carcinoma: Long-term follow-up of 990 cases. Obstet Gynecol 37:1–9, 1977.
2. Kapp DS: The role of the radiation oncologist in the management of gynecologic cancer. Cancer 51:2485–2497, 1983.
3. Fletcher GH: Clinical dose-response curves of human malignant epithelial tumors. Brit J Radiol 46:1–12, 1973.
4. Rubin P: Understanding the problem of understaging in ovarian cancer. Semin Oncol 2:235–242, 1975.
5. Fletcher GH: Clinical dose-response curve of subclinical aggregrates of epithelial cells and its practical application in the management of human cancers. In: Biological and Clinical Basis of Radiosensitivity, Friedman M (ed), Springfield, Illinois, Charles C. Thomas (1974) pp 485–501.
6. Santesson L, Kottmeier HL: General classification of ovarian tumors. In: Ovarian Cancer, Gentils F, Junguerra AC (eds). UICC Monograph Series, Vol 11, New York, Springer-Verlag (1968) pp 1–8.

7. Griffiths CT, Fuller AF: Intensive surgical and chemotherapeutic management of advanced ovarian cancer. Surg Clin North Am 58:131–142, 1978.
8. Griffiths CT, Parker LM, Fuller AF: Role of cytoreductive surgical treatment in the management of advanced ovarian cancer. Cancer Treat Rep 62:235–240, 1979.
9. Eymer H: Bleinflusoring von proliferierender ovariant tumoren durch roentgenstrahlen. Strahlentherapie 1:358–361, 1912.
10. Delclos L, Dembo AJ: Ovaries. In: Textbook of Radiotherapy, Fletcher GH (ed) Third Edition, Philadelphia, Lea and Febiger (1980) pp 834–851.
11. Delclos L, *et al.*: Whole abdominal irradiation by ^{60}Co moving strip technique. Radiol 81:632, 1963.
12. Smoron GL: Strip-Staggering: Elimination of in homogeneity in the moving strip technique of whole abdominal radiation. Radiol 104:657–660, 1972.
13. Fazekas JT, Maier JG: Irradiation of ovarian carcinomas: A prospective comparison of the open-field and moving strip techniques. Am J Roentgenol 120:118–123, 1974.
14. Dembo AJ: The role of radiotherapy in ovarian cancer. Bullentin du Cancer 69:275–284, 1982.
15. Brady LW: Cancer of the ovary. In: Modern Radiation Oncology Classic Literature and Current Management, Gilbert HA, Kagan AR (eds), Hagerstown, Maryland, Harper and Row (1978) pp 582–609.
16. Martinez A: Perspective: The role of radiation therapy in the treatment of epithelial ovarian cancer. In: Gynecologic Oncology, Controversies in Cancer Treatment, Ballon SC (ed), Boston, Massachusetts, Hall (1981) pp 300–310.
17. Arcameli G, Benassi M, DePera V, *et al.*: A new fractionation technique of total abdominal irradiation in ovarian cancer. Int J Rad Oncol Biol Phys 1:155–160, 1975.
18. Muller JH: Uber die verwendung von Kunstlichen radioaktiven isotopen zur erzielung von lokalisierten biologischen strahlenwirkungen. Experientia 1:199–200, 1945.
19. Francis HC, Hahn PF, Goodall JPB, *et al.*: Direct infiltration of radioactive isotopes as means of delivering ionizing radiation to discrete tissues. J Lab Clin Med 32:1442, 1947.
20. Muller JL: Weitere entwicklungen der Therapie von peritoneal carcinosen bei ovarialcarcinon mit kunstlicher radioaktivitat (^{198}Au). Gynecologia 129:189–294, 1950.
21. Muller JH: Curative aim and results of routine intraperitoneal radiocolloid administration in the treatment of ovarian cancer. Am J Roent Rad Ther Nucl Med 89:533–540, 1963.
22. Keettel WC, Elkins HB: Experience with radioactive colloidal gold in the treatment of ovarian carcinoma. Am J Obstet Gynecol 71:553–568, 1956.
23. Card RY, Cole DR, Henschke UK: Summary of ten years of the use of radioactive colloids in intracavitary therapy. J Nucl Med 1:195–198, 1960.
24. Holbrook MD, Welch JS, Childs DS: Adjuvant use of radioactive colloids in treatment of carcinoma of the ovary. Radiol 83:888–891, 1964.
25. Keettel WC, Fox MC, Longnecker DS, Latourette HB: Prophylactic use of radioactive gold in the treatment of primary ovarian cancer. Am J Obstet Gynecol 94:766–775, 1966.
26. Clark DGC, Hilaris B, Roussis C, Brunschwig A: The role of radiation therapy (including isotopes) in the treatment of cancer of the ovary (results of 614 patients at Memorial Hospital, New York). Clinical Cancer 5:227–235, New York: Grune & Stratton, 1973.
27. Decker DG, Webb MJ, Holbrook MA: Radiogold treatment of epithelial cancer of the ovary. Am J Obstet Gynecol 115:751–756, 1973.
28. Kolstad P, Davy J, Hoeg K: Individualized treatment of ovarian cancer. Am J Obstet Gynecol 128:617–625, 1977.
29. Dillman LT, Vanderlage FC: MIRD pamphlet No 10. J Nucl Med 4:20–104, 1971.
30. Leighton RB: Principles of Modern Physics, New York: McGraw-Hill, 1965.
31. Pizzarello DJ, Witcofski RL: Basic Radiation Biology, Philadelphia: Lea & Febiger, 1970.
32. Jones HB, Wrobel CJ, Lyons WR: A method of distributing beta-radiation to the reticulo-

endothelial system and adjacent tissue. J Clin Invest 28:783–788, 1944.
33. Morton ME: Colloidal chromic radiophosphate in high yields for radiotherapy. Nucleonics 92:96, 1952.
34. Henschke UK, Hilaris BS, Mahan GD: Afterloading in interstitial and intracavitary radiation therapy. Am J Roentgeno 90:385–395, 1963.
35. Ariel JM, Oropeza R, Pack GT: Intracavitary administration of radioactive isotopes in the control of effusion due to cancer. Cancer 19:1096–1102, 1966.
36. Hester LL, White L: Radioactive colloidal chromic phosphate in the treatment of ovarian malignancies. Am J Obstet Gynecol 103:911–918, 1969.
37. Piver MS, Barlow JJ, Lele SB: Incidence of subclinical metastasis in stage I and II ovarian cancer. Obstet Gynecol 52:100–104, 1978.
38. Van den Brenk HAS, Clarke KH, Holman WP, Winkler C: Studies of the effect of a colloidal radioactive chromic phosphate ($Cr^{32}PO_4$) in clinical and experimental malignant effusions. Brit J Cancer 13:181–194, 1959.
39. Silver S: Radioactive Isotopes in Medicine and Biology, Philadelphia: Lea & Febiger, 1962.
40. Blahd WH: Nuclear Medicine, New York: McGraw-Hill, 1965.
41. Root SW, Tyor MP, Andrews GA, Kniselay RM: Distribution of colloidal radioactive chromic phosphate after intracavitary administration. Radiol 63:251–259, 1954.
42. Johns HE, Cunningham JR: The physics of Radiology, Springfield: Charles C. Thomas, 1969.
43. Leichner PK, Cash SA, Backa C, Durand R: Effect of injection volume on the tissue dose, dose rate, and therapeutic potential of intraperitoneal ^{32}P. Radiol 141:193–199, 1981.
44. Horowitz DA: Technic of intraperitoneal instillation of radioactive colloids. Obstet Gynecol 42:621–622, 1973.
45. Alderman SJ, Dillon TF, Krummerman MS, *et al.*: Postoperative use of radioactive phosphorus in stage I ovarian carcinoma. Obstet Gynecol 49:659–662, 1977.
46. Taylor A, Baily NA, Halpern SE, Ashborn WL: Loculation as a contraindication to intracavitary ^{32}P-chromic phosphate. J Nucl Med 16:318–319, 1975.
47. Tully TE, Goldberg ME, Loken MK: The use of $99^{m}Tc$-sulfur colloid to assess the distribution of ^{32}P-chromic phosphate. J Nucl Med 16:318–319, 1974.
48. Goldie H, Hahn PF: Distribution and effect of colloidal radioactive gold in peritoneal fluid containing free sarcoma-37 cells. Proc Soc Experimental Biol Med 74:638–642, 1950.
49. Dobson EL, Jones HB: The behaviour of intravenously injected particulate material. Acta Medica Scand 144(Suppl):273, 1951.
50. Cooper JA, Lorn EM, Hines I: Distribution of colloidal radioactive chromic phosphate after intracavitary administration in the rat. J Lab Clin Med 42:867–871, 1953.
51. Vider M, Deland FH, Maruyama Y: Loculation as a contraindication to intracavity ^{32}P-chromic phosphate therapy. J Nucl Med 17:150–151, 1976.
52. Heidelberg JG, Sirota PG, Dewey WC, Rose RG: Determination of the distribution of ^{32}P in phantom limb patients and in patients by detection of Bremsstrahlung radiation with a scimtiscanner. Am J Roentgen 90:325–335, 1963.
53. Kaplan WD, Zimmerman RE, Bloomer WD, *et al.*: Therapeutic intraperitoneal ^{32}P: a clinical assessment of the dynamics of distribution. Radiol 138:683–688, 1981.
54. Leichner PK, Rosenshein NB, Leibel SA, Order SE: Distribution and tissue dose of intraperitoneally administered radioactive chromic phosphate (^{32}P) in New Zealand white rabbits. Radiol 134:729–734, 1980.
55. Currie JL, Bayne F, Harris C, *et al.*: Radioactive chromic phosphate suspension: Studies on distribution, dose absorption and effective therapeutic radiation in phantoms, dogs & patients. Gynecol Oncol 12:193–218, 1981.
56. Twefik HH, Gruber H, Tewfik FA, Lifshitz SG: Intraperitoneal distribution of ^{32}P-chromic phosphate suspension in the dog. Int J Rad Oncol Biol Phys 5:1907–1913, 1979.

57. Myers MA: Peritoneography. Normal and pathologic anatomy. Am J Roentgen Rad Ther Nucl Med 117:353–365, 1973.
58. Buchsbaum HJ, Keettel WC, Latovrette HB: The use of radioisotopes as adjunct therapy of localized ovarian cancer. Semin Oncol 2:247–251, 1975.
59. Rosenshein N, Blake D, McIntyre PA, *et al.*: The effect of volume on the distribution of substances into the peritoneal cavity. Gynecol Oncol 6:106–110, 1978.
60. Villasanta U, Bloedorn FG: Operation, external irradiation radioactive isotopes and chemotherapy in treatment of metastatic ovarian malignancies. Am J Obstet Gynecol 102:531–536, 1968.
61. Pezner CA, Stevens KR, Tong D, Allen CV: Limited epithelial carcinoma of the ovary treated with curative intent by the intraperitoneal instillation of radiocolloids. Cancer 42:2563–2571, 1978.
62. Dembo AJ, Bush RS: Radiation therapy of ovarian cancer. In: Gynecologic Oncology, Griffiths CT and Fuller A (eds) Boston, M. Nijjoff (1983) pp 263–298.
63. Dembo AJ, Bush RS, Beale FA, *et al.*: The Princess margaret study of ovarian cancer: Stages I, II and asymptomatic III presentation. Cancer Treat Rep 63:249–254, 1979.
64. Hreshchyshyn MM, Park RC, Blessing JA, *et al.*: The role of adjuvant therapy in Stage I ovarian cancer. Am J Obstet Gynecol 138:139–145, 1980.
65. Smith JP, Rutledge FN, Delclos L: Postoperative treatment of early cancer of the ovary: A random trial between postoperative radiation and chemotherapy. Natl Cancer Inst Mongraph 42:149–153, 1975.
66. Young RC: A strategy for effective management for early ovarian cancer. In: Adjuvant Therapy of Cancer, II, Jones Se, Salmon SE (eds) New York, Grune and Stratton (1979) pp 467–474.
67. Bagley CM, Young RC, Canellos GP, DeVita VT: Treatment of ovarian carcinoma: Possibilities for progress. N Engl J Med 287:856–862, 1972.
68. Tobias JS, Griffiths CT: Management of ovarian cancer. New Engl J Med 294:818–823, 1976.
69. Buchsbaum HJ, Keettel WC: Radioisotopes in treatment of stage Ia ovarian cancer. Symposium of ovarian cancer. Natl Cancer Inst Monograph 42:127–128, 1975.
70. Moore DW, Langley H: Routine use of radiogold following operating for ovarian cancer. Am J Obstet Gynecol 98:624–628, 1967.
71. Perez CA, Walz BJ, Jacobson PL: Radiation therapy in the management of carcinoma of the ovary. Symposium of ovarian cancer. Natl Cancer Inst Monograph 42:119–125, 1975.
72. Rose RG: Intracavitary radioactive colloidal gold in the management of ovarian carcinoma. Obstet Gynecol 18:557–563, 1961.
73. Fletcher GH: Textbook of Radiology, Philadelphia: Lea and Febiger, 1973.
74. Julian JC, Inalsingh CHA, Burnett LS: Radioactive phosphorous and external radiation as an adjuvant to surgery for ovarian carcioma. Obstet Gynecol 52:155–160, 1978.
75. Piver MS, Barlow JJ, Lele SB, *et al.*: Intraperitoneal chromic phosphate in peritoneoscopically confirmed stage I ovarian adenocarcinoma. Am J Obstet Gynecol 144:836–840, 1982.
76. Dembo AJ, Bush RS, Beale FA, *et al.*: Ovarian carcinoma: Improved survival following abdominopelvic irradiation in patients with a completed pelvic operation. Am J Obstet Gynecol 134:793–800, 1979.
77. Dembo Aj: Radiation therapy in the management of ovarian cancer. Clinics Obstet Gynecol 10(2):261–278, 1983.
78. Brady LW, Blessing JA, Slayton RE, *et al.*: Radiotherapy, chemotherapy, and combined modality therapy in stage IV epithelial ovarian cancer. Cancer Clin Trials 2:111–120, 1979.
79. Schray MF, Cox R, Martinez A: Lower abdominal radiotherapy for stage I, II and selected III epithelial ovarian carcinoma: 20 year experience. Gynecol Oncol 15:78, 1983.

80. Schray M, Martinez A, Cox R: Radiotherapy in epithelial ovarian cancer: Analysis of prognostic factors based on long term experience. Obstet Gynecol 62:373–382, 1983.
81. Hainsworth JD, Malcolm A, Johnson DH, *et al.*: Advanced minimal residual ovarian carcinoma: Abdominopelvic irradiation following combination chemotherapy. Obstet Gynecol 61:619–623, 1983.

7. Single agent chemotherapy in the management of ovarian carcinoma

J. TATE THIGPEN

Ovarian cancer is comprised of celomic epithelial cancers, germ cell neoplasms, stromal cancers, and certain other rare lesions. Celomic epithelial lesions account for over 85% of all ovarian cancers; therefore, most data concerning the management of ovarian cancer relate to studies in patients with ovarian carcinoma arising from celomic epithelium. Discussion of single-agent systemic therapy will thus relate only to these lesions of celomic epithelial origin (henceforward referred to simply as ovarian carcinoma).

The importance of systemic therapy in the management of these ovarian carcinomas stems from the facts that they tend to present at an advanced stage and that they respond with a high frequency to systemic agents. Whereas previously such therapy was regarded as palliative only, recent results with combination chemotherapy suggest that cure is a feasible goal. The following discussion will examine single systemic agents in the management of patients with advanced or recurrent ovarian carcinoma and will focus on: (1) cytotoxic drugs of proven efficacy, (2) cytotoxic drugs with probable efficacy, (3) cytotoxic agents with no apparent activity, and (4) hormonal agents of possible value. These considerations will serve as a prelude to discussion of combination chemotherapy in ovarian carcinoma.

1. General considerations

The efficacy of systemic chemotherapy in ovarian carcinoma is determined to a major extent by certain factors other than the agent to be administered: histologic type and grade, extent of disease, and prior therapy. In regard to histologic type, ovarian carcinomas of celomic origin are divided into 5 categories: serous, mucinous, endometrioid, mesonephroid, and undifferentiated. There is little evidence to support a relationship between histologic type and response to cytotoxic drugs, but there may well be a greater likelihood that serous and endometrioid carcinomas will respond to

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hormonal agents [1, 2]. Histologic grade, on the other hand, may be extremely important in determining response to chemotherapy [3–5]. Well-differentiated lesions of low malignant potential ('borderline' carcinomas characterized by no stromal invasion) have a generally favorable outlook regardless of therapy. For those lesions other than 'borderline' carcinomas, degree of differentiation exerts a major influence on prognosis and, in at least one study, on response to chemotherapy [5].

Extent of disease plays a major role in determining the efficacy of chemotherapy. In those patients with advanced or recurrent disease, in whom most single agents have been studied, the extent of disease remaining after surgery has been shown to exert a major influence on the frequency with which objective regression of disease has been observed. Those stage III patients with no residual nodule greater than 2 centimeters in diameter exhibit an 84% objective response rate to chemotherapy as compared to only 53% in those with bulkier (greater than 2 centimeter nodules or stage IV) disease (Table 1) [6]. Survival is influenced in a similar fashion by residual bulk of disease in patients with advanced or recurrent ovarian carcinoma treated with melphalan (Table 2) [7, 8].

Previous therapy to which the patient has been exposed will also determine to a great extent the patient's chances of response to chemotherapy. A number of studies have shown a lower respone to chemotherapy in those patients who had received prior chemotherapy than in those with no such prior exposure [9–15]. Some evidence also suggests a lower response rate in patients who have received prior radiotherpay [16].

All of these factors as well as other less well-defined influences such as the nutritional status of the patient must be considered when the efficacy of systemic agents is evaluated in patients with ovarian carcinoma. For older,

Table 1. The relation between extent of disease and response to chemotherapy in patients with advanced or recurrent ovarian carcinoma [6]

Disease	Response
Stage III with residual nodules ≤ 2 cm	16/19 (84%)
Stage IV and Stage III with residual nodules > 2 cm	31/58 (53%)

Table 2. The relation between extent of disease and survival in patients with advanced or recurrent ovarian carcinoma treated with mephalan [7, 8]

Disease	Median survival
Stage III with residual nodules ≤ 3 cm [8]	33 mos
Stave IV and Stage III with residual nodules > 3 cm[7]	12 mos

more established drugs, not all of these details are available; hence estimation of the level of efficacy can be difficult. Subsequent use of some of these agents in control arms of trials of combination chemotherapy has provided us with better estimates of efficacy. With these limitations in mind, it is now reasonable to examine systemic agents studied in patients with ovarian carcinoma.

2. Cytoxic drugs of established efficacy

Cytoxic drugs used as single agents were considered to be standard therapy for advanced or recurrent ovarian carcinoma until recently. Those agents accepted as having the greatest efficacy and hence most commonly used include: alkylating agents, cisplatin, adriamycin, hexamethylmelamine, 5-fluorouracil, and methotrexate [17-23]. These are currently the principal agents in effective combination chemotherapy and hence deserve detailed consideration as single agents in the management of ovarian carcinoma.

2.1. Alkylating agents

Alkylating agents have been the most commonly used single agents in the management of ovarian carcinoma. The particular drugs for which substantial data exist include (Table 3): melphalan (Alkeran, L-phenylalanine mustard, L-PAM), cyclophosphamide (Cytoxan), chlorambucil (Leukeran), nitrogen mustard (Mustargen, mechlorethamine), and thiotepa (triethylenethiophosphoramide) [17–20]. No substantial differences among these in regard to efficacy have been proven. Melphalan has been and still is the most commonly used alkylating agent when single-drug therapy is given; hence, a more detailed review of melphalan is warranted to be followed by a brief consideration of the other alkylators.

Melphalan is an analogue of nitrogen mustard formed from phenylalanine

Table 3. Alkylating agents used as single drugs in the management of patients with advanced or recurrent ovarian carcinoma [20]

Drugs	Patients	Response rate
Melphalan [20]	494	47%
Cyclophosphamide [20]	262	44%
Chlorambucil [20]	390	51%
Nitrogen mustard [20]	81	31%
Thiotepa [20]	144	64%

and the parent compound. Its relative freedom from the acute nausea and vomiting and alopecia noted with nitrogen mustard and its oral route of administration probably account for its common use as the alkylating agent for patients with ovarian carcinoma. The most frequently reported schedule is 0.2 mg/kg/day orally for 5 days repeated every 4 to 6 weeks as tolerated [24]. Extended treatment periods of 12 to 24 months in patients with responsive neoplasms or with no measurable lesions are usual. Adverse effects under such circumstances include: myelosuppression (leukopenia and thrombocytopenia) with a nadir most frequently 14 to 21 days after initiation of a course of therapy but occasionally delayed to 35 to 40 days; infrequent alopecia, dermatitis, stomatitis, and pulmonary fibrosis [25]; and increased frequency of acute leukemia usually associated with long-term administration [26] and most commonly myelocytic and myelomonocytic [27].

The reported frequency of objective response of ovarian carcinoma to melphalan varies significantly from a low of 12% [28] to a high of 64% [29]. The reasons for this striking variation are not entirely clear but probably include: a variable number of patients with optimal stage III disease in each study population, a variable number of patients with prior radiotherapy and/or chemotherapy, differences in response criteria, and variation in absorption of melphalan from the gastrointestinal tract by as much as fourfold [30]. A more precise estimate of the level of efficacy of melphalan can be obtained by a review of the experience of the Gynecologic Oncology Group with the drug given at the dose and schedule noted above to patients who had at time of drug initiation suboptimal stage III (nodules >3 cm diameter remaining), stage IV, or recurrent ovarian carcinoma, had received no prior radiotherapy or chemotherapy, and were judged by uniform response criteria (Table 4) [31–33]. In 3 separate protocols, melphalan control arms yielded response rates which were notably consistent (Table 5). The facts that the study populations were uniform and included no optimal stage III disease, that the numbers of patients studied were relatively large,

Table 4. Definitions of response to therapy commonly used in current trials

Complete Response (CR): Disappearance of all evidence of gross disease for at least 1 month
Partial Response (PR): 50% or greater reduction in the product of perpendicular diameters of each measurable lesion and no new lesions for at least 1 month
Stable Disease (SD): Less than 50% increase or decrease in measurable disease and no new lesions for at least 1 month
Increasing Disease (ID): 50% or greater increase in the product of perpendicular diameters of any measurable lesion or the appearance of any new lesion within 1 month of initiation of therapy

Table 5. Response rates for melphalan* in the treatment of advanced ovarian carcinoma in three GOG trials

GOG study	Patients	Complete response	Partial response
Protocol 2 [31]	40	5 (13%)	8 (20%)
Protocol 3 [32]	93	15 (16%)	12 (13%)
Protocol 22 [33]	60	10 (17%)	12 (20%)
Composite	193	30 (16%)	32 (17%)

* Melphalan 0.2 mg/kg/day for 5 days every 4–6 weeks.

and that response criteria were uniform strongly suggest that, in patients with bulky advanced disease, melphalan can be expected to achieve an objective response in 33% of patients, about half of whom will achieve a clinically complete response. The median duration of response was 7 months with a median survival for all treated patients of 12 months. Responders survived significantly longer than non-responders.

No other alkylating agents have been studied in so uniform a fashion as melphalan. That these other alkylators are active is evident from data cited (Table 3). No adequate study to compare the efficacy of these agents has been done. Attempts at such a project [28, 29] have yielded insignificant differences in small numbers of patients.

In summary, certain alkylating agents possess definite activity against ovarian carcinoma. One can expect objective regressions in approximately 33% of previously untreated patients with bulky advanced disease, about half of which will be clinically complete. While median response duration is only 7 months and median survival only 12 months for patients so treated, it should be noted that a small percentage of these patients (5–10%) will be alive 5 years after diagnosis [34–36]. Melphalan, although it is the most commonly used and studied agent, offers no clear advantage over other alkylating agents and in fact may be disadvantageous because of its variable absorption from the gastrointestinal tract.

2.2. Cisplatin

Cisplatin is a heavy metal complex with an incompletely defined mechanism of action. The drug is usually administered in a dose of 50 to 120 mg/m^2 intravenously at 3 to 4 week intervals. Major adverse effects include: a dose-related nephrotoxicity manifested by renal tubular damage which results in azotemia, is reversible, and can be prevented by aggressive hydration and mannitol diuresis [37]; ototoxicity manifested by high-frequency, subclinical hearing loss progressing to clinically evident effects if the

drug is continued [38]; anaphylactic reactions consisting of tachycardia, wheezing, hypotension, and facial edema [38]; minimal to moderate myelosuppression with a nadir at 2 weeks after drug administration [38]; peripheral neuropathies [38]; moderate to severe nausea, vomiting, and diarrhea [38]; and other, uncommon effects such as hyperuricemia and transient elevation of liver enzymes. Dose is usually limited by either nephrotoxicity or neuropathy.

A number of trials of cisplatin as a single agent in ovarian carcinoma have been carried out with response rates which range from 5% to 52% (Table 6) [21, 22, 39–43]. All but one of these trials was carried out in patients refractory to standard alkylating agents; hence the composite response rate of 32% is surprising and suggests significant activity on the part of cisplatin. As would be expected with second-line single-agent therapy, most responses were partial and short in duration; but occasional, durable complete responses were observed. The optimum dose and schedule for the drug cannot be determined from available data, but a majority of the patients received 50 to 75 mg/m^2 intravenously administered as a single dose over 1 to 2 hours every 3 weeks as tolerated.

Whether more aggressive schedules will yield even better results is not known. One recent trial suggests that such may be the case [44]. Investigators at the Royal Marsden Hospital administered cisplatin at either 30 mg/m^2 or 100 mg/m^2 every 3 weeks to 82 patients who had received previous chemotherapy. Response rates of 27% and 52% respectively were observed. In the low-dose regimen, 6% achieved a clinically complete response, whereas 16% achieved a complete response in the high-dose arm. These results are clouded by two factors. First, 23 of the patients on the low-dose arm actually received 90 mg/m^2 per course (30 mg/m^2 daily for 3

Table 6. Cisplatin as a single agent in previously treated patients with ovarian carcinoma

Investigator	Dose and schedule	Patients	Response rate
Bruckner et al. [41]*	50 mg/m^2 every 3 weeks	17	35%
Bruckner et al. [42]	50 mg/m^2 every 3 weeks	19	31%
Piver et al. [43]	50–100 mg/m^2 every 3 weeks	20	5%
Rossof et al. [40]	75 mg/m^2 every 3 weeks	15	13%
Thigpen et al. [21]	50 mg/m^2 every 3 weeks	37	24%
Wiltshaw et al. [39, 44]	30 mg/m^2 day 1 every 3 weeks	29	28%
	30 mg/m^2 days 1–3 every 4 weeks	23	26%
	100 mg/m^2 every 3 weeks	30	52%
Overall		190	32%

* No prior chemotherapy.

days every 4 weeks) with no observable difference in response between this group and those who received only 30 mg/m^2 one time every 3 weeks. Secondly, the trial was not randomized, nor were enough patients studied to permit valid comparisons. The issue of optimum dose and schedule thus remains unanswered.

In summary, cisplatin appears to be significantly active against ovarian carcinoma. The level of efficacy appears similar to that observed with standard alkylating agents. Optimum dose and schedule for the drug remain unclear at present. Current studies concern primarily platinum-based combination chemotherapy and certain innovative approaches to administration of extremely aggressive cisplatin schedules (discussed elsewhere in this volume).

2.3. Adriamycin

Adriamycin is an antibiotic obtained from a streptomyces species and is a hydroxylated congener of daunorubicin. It appears to exert its effect by intercalation between adjoining nucleotide pairs in DNA [45] and hence to prevent DNA-dependent RNA synthesis. Adverse effects commonly seen include: acute, dose-limiting myelosuppression principally manifested as leukopenia with a nadir at 10 to 14 days; local tissue damage at site of extravasation; marked alopecia; stomatitis; nausea, vomiting, and diarrhea; radiosensitization and recall phenomena in areas of previous radiation; and cardiotoxicity. The last effect is of the greatest concern and includes acute toxicities such as acute inflammation and/or injury and acute arrhythmias as well as an intractable cardiomyopathy associated with an increased frequency after a total cumulative dose of greater than 550 mg/m^2. Drug courses are usually administered at 3-week intervals in doses from 60 to 90 mg/m^2 intravenously.

As a single agent, adriamycin has been administered to 102 patients with advanced or recurrent ovarian carcinoma [20, 46–50]. Dose and schedule in each case followed generally accepted guidelines noted above. From available data, the number of patients who had received no other chemotherapy prior to receiving adriamycin is not clear; nor is it clear how many had previously received radiotherapy. That adriamycin possesses significant activity against ovarian carcinoma is clear in that 34 of 102 patients (33%) treated did manifest an objective regression of disease of significant (> 50%) magnitude. Furthermore, in at least one study, responses to adriamycin were observed in patients whose tumors were refractory to melphalan [47]. Adriamycin would thus appear to be a potentially useful drug for combinations including alkylating agents and cisplatin.

2.4. Hexamethylmelamine

Hexamethylmelamine is a melamine derived from cyanuric chloride and is structurally similar to an alkylating agent triethylenemelamine (TEM). Its exact mechanism of action is, however, not clear [51]. Certain studies suggest that it may not be cross-resistant with standard alkylating agents [52, 53]. Common adverse effects include: dose-limiting gastrointestinal toxicity manifested by anorexia, nausea, vomiting, diarrhea, and abdominal cramps [54]; neurotoxicity manifested by peripheral neuropathies and central nervous system effects such as agitation, confusion, hallucinations, depression, Parkinsonian symptoms, and petit-mal seizures; and mild leukopenia and trhombocytopenia. The drug is usually given in daily oral doses of 4 to 12 mg/kg/day for 21 days repeated every 6 weeks.

The use of hexamethylmelamine as a single agent in ovarian carcinoma is summarized in a review by Weiss [55]. The drug appears to possess activity both in previously untreated patients and in patients whose cancers are resistant to standard alkylating agents (Table 7) [53, 54, 56–62]. Among responding patients are a number of patients (15% in one study) who achieved a clinically complete response which proved to be durable and, in two instances, a pathologically complete response documented by a second-look laparotomy [56]. This demonstrated efficacy plus apparent lack of absolute cross resistance with alkylating agents make hexamethylmelamine an attractive drug for combination trials, some of which are underway at the present time.

2.5. 5-Fluorouracil

5-Fluorouracil is a fluorinated pyrimidine which exerts its cytotoxic effect by acting as an antimetabolite to inhibit the formation of thymidine. This inhibition appears to result from competition between the drug and the natural substrate uracil deoxyribotide for the enzyme thymidylate synthetase. Adverse effects commonly seen include: myelosuppression manifested

Table 7. Hexamethylmelamine as a single agent in the management of advanced or recurrent ovarian carcinoma

Category	Patients	Response rate
No prior chemotherapy [56]	54	31%
Prior chemotherapy unspecified [54, 57]	22	36%
Prior chemotherapy [53, 58–62]	139	19%
Overall	215	24%

as leukopenia and thrombocytopenia with a nadir between 7 and 14 days after initiation of a drug course; gastrointestinal effects including nausea, vomiting, diarrhea, stomatitis, esophagitis, and proctitis; dermatologic effects such as photosensitivity, rashes, and alopecia; acute cerebellar ataxia with or without headache and visual disturbance; hypotension; and hyperpigmentation over veins through which the drug has been administered. Drug dose and schedule vary significantly from study to study; favored schedules are a bolus of 12 mg/kg daily for 4 days or a 4-day continuous infusion of 1000 mg/m^2/day. These can be followed either by a weekly maintenance or by repeated 4-day courses at 4–6 weeks intervals.

The use of 5-fluorouracil as a single agent in patients with ovarian carcinoma has been reported in a number of series [63–71]. Overall, 37 objective responses were observed among 126 patients treated. A majority of these patients had received no prior chemotherapy. The overall 29% response rate suggests that the drug has significant activity against ovarian carcinoma. Responses were observed regardless of dose and schedule selected. Most patients did receive an initial 5 day course of 15 mg/kg/day of the drug followed by alternate day maintenance, but a 33% response rate was observed among 21 patients treated with a weekly schedule [71]. The apparent efficacy of 5-fluorouracil plus its differing mechanism of action as compared to other known active drugs make this agent a prime candidate for combination chemotherapy trials.

2.6. Methotrexate

Methotrexate is a folic acid antagonist which binds to dihydrofolate reductase and thus blocks the reduction of dihydrofolate to tetrahydrofolic acid. The ultimate result of this is to block the synthesis of thymidine and hence to block DNA synthesis. Adverse effects seen include: myelosuppression affecting all elements with a nadir usually within one week followed by rapid recovery; gastrointestinal effects consisting of not only nausea, vomiting, and diarrhea but also ulceration of gastrointestinal tract surface membranes from mouth to anus; hepatotoxicity manifested as both acute hepatocellular injury and delayed fibrosis and/or cirrhosis associated with prolonged therapy; a variety of dermatologic effects including rashes and alopecia; encephalopathy; and renal failure as a result drug precipitation in tubules in an acidic environment. Drug dose and schedule may vary and depend to some extent on whether rescue of normal tissue with leucovorin is employed.

Methotrexate has been used as a single agent in patients with ovarian carcinoma in a relatively small number of patients [72, 73]. Among a total of 26 patients in two studies using standard doses of methorexate, 5 objec-

tive responses, all partial, were observed. A series of 19 patients with advanced ovarian carcinoma received a higher dose schedule of 1 to 7.6 gm/m^2 of methotrexate weekly with rescue with citrovorum factor and adequate hydration with alkalinization [74]. Among 8 patients with measurable disease, only one partial response was observed after 6 to 12 weeks of treatment. Among the 11 patients with non-measurable disease, obvious progressive disease was observed in 4 after a similar period of treatment. It should be noted that all patients had received prior chemotherapy. From these data, it can be concluded that methotrexate does possess moderate activity against ovarian carcinoma at least in lower-dosage schedules. The higher dosage schedule cannot be evaluated from present data. Because of its differing mechanism of action from other active agents, methotrexate at low dose would thus seem to be an attractive possibility for combination chemotherapy despite the relative paucity of patients treated with the drug as a single agent.

2.7. Summary of established active agents

There are a relative abundance of active drugs in ovarian carcinoma (Table 8). Use of these as single agents can be reasonably expected to produce objective regressions in one out of three patients with suboptimal stage III (nodules > 3 cm diameter) and stage IV or recurrent ovarian carcinoma who have not been exposed to prior chemotherapy. Approximately half of these responses will be clinically complete responses with significantly prolonged response duration and survival, and a small proportion of patients so treated will be pathologically free of disease with extremely long disease-free intervals and perhaps, in some instances, cure. Typical dose and schedule for these agents employed as single-drug therapy are summarized in Table 9. In most instances, optimum dose and schedule as well as optimum duration of therapy are not clear from available data.

Table 8. Summary of drugs of established efficacy in the management of advanced (stage III or IV) or recurrent ovarian carcinoma

Drug	Patients	Response rate
Alkylating agents [20]	1371	31–64%
Cisplatin [21, 22, 39, 44]	190	32%
Adriamycin [20, 46–50]	102	33%
Hexamthylmelamine [53–62]	215	24%
5-Fluorouracil [19, 63–71]	126	29%
Methotrexate [20, 72–74]	34	18%

Table 9. Typical dose and schedule employed in single agent therapy for advanced or recurrent ovarian carcinoma

Drug	Dose and schedule
Melphalan	0.2 mg/kg/day p.o. days 1–5 repeated every 4–6 weeks as tolerated
Cisplatin	50–100 mg/m^2 IV day 1 repeated every 3–4 weeks as tolerated
Adriamycin	60–90 mg/m^2 IV day 1 repeated every 3 weeks to maximum cumulative dose of 550 mg/m^2
Hexamethylmelamine	4–12 mg/kg/day p.o. days 1–21 repeated every 6 weeks
5-Fluorouracil	12 mg/kg/day IV days 1–5, then 6 mg/kg/every other day IV, to toxicity, repeat every 3-6 weeks
Methotrexate	5 mg/day p.o. or IV for 5 to 10 days repeated every 3–4 weeks

More important than considerations for the use of these drugs as single agents, however, is the potential that exists for effective combination chemotherapy. The active drugs fall into 6 different categories according to presumed mechanism of action and hence provide excellent potential for combination chemotherapy, a potential that is enhanced further by the relatively less myelosuppression seen with cisplatin and hexamethylmelamine. Such an approach offers greater hope of increased frequency and duration of responses than further exploitation of single-agent therapy and will be discussed in a subsequent chapter.

3. Cytotoxic drugs with probable efficacy

Although, as has been discussed, there is a relative abundance of active drugs in the treatment of ovarian carcinoma, even effective combinations of these drugs fail to cure a majority of patients with advanced or recurrent ovarian carcinoma [6, 75]. There is thus a significant need to develop new additions to our therapeutic armamentarium in the hopes that the level of efficacy of primary treatment can be further enhanced. Studies of a number of newer agents, most of which are not yet commercially available, have provided suggestive evidence of activity for several of these compounds. These potentially active new agents will now be discussed.

3.1. Platinum analogues

As noted earlier, cisplatin is a coordination complex of platinum which has considerable activity against ovarian carcinoma at the expense of significant toxicity. In an effort to increase efficacy and decrease toxicity, a number of platinum analogues have been developed [76–78]. One of these analogues, cis-diammine-1, 1-cyclobutane dicarboxylate platinum II (carboplatin, CBDCA, JM8, NSC 241240), in preclinical studies has retained significant antitumor activity comparable to that seen with cisplatin [76, 77] with less nephrotoxicity [78]. Phase I trials have subsequently shown myelosuppression to be the dose-limiting toxicity with no evidence of significant renal or ototoxicity and only mild to moderate nausea and vomiting [79]. The decrease in toxicity with apparently equivalent efficacy makes carboplatin a potentially attractive alternative to cisplatin.

A recent clinical trial in Great Britain compared carboplatin to cisplatin in previously untreated patients with ovarian carcinoma [80]. The rationale for the study was based on data from two unpublished trials cited by Wiltshaw and colleagues [81], the first a phase II study of carboplatin in ovarian carcinoma suggesting a high but unspecified order of activity in 70 patients whose prior treatment history was not given and the second a comparative trial of cisplatin with or without chlorambucil showing no advantages to the combination. Although the number of patients involved at the time of the report was too small to permit definite conclusions, preliminary data suggest similar response rates with much less toxicity in the patients treated with carboplatin (Table 10). Nephrotoxicity was defined as a 20% decrease in creatinine clearance. Ototoxicity consisted of deterioration on repeat audiogram. Cisplatin therapy was given as 5 monthly doses of 100 mg/m^2 fol-

Table 10. Results of a trial of cisplating versus carboplatin in ovarian carcinoma [80]

Parameter	Cisplatin	Carboplatin
Objective Response		
Complete response	3 (14%)	3 (17%)
Partial response	8 (38%)	6 (33%)
No response	10 (48%)	9 (50%)
Not evaluable	5	4
Adverse effects		
Nephrotoxicity	71%	12%
Ototoxicity	65%	0%
Peripheral neuropathy	19%	0%
Leukopenia (<3000/mcl)	31%	36%
Thrombocytopenia (<100.000/mcl)	0%	9%

lowed by five monthly doses of 20 mg/m^2, whereas carboplatin was given as 10 monthly doses of 400 mg/m^2.

A second platinum analogue of interest is cis-dichloro-trans-dihydroxy-bis-(isopropylamine)-platinum (IV) (CHIP, NSC256927) [76, 77]. Phase I studies [82] suggest a decrease in renal and gastrointestinal adverse effects similar to that seen with carboplatin. No comment can be made at present about level of efficacy.

Both of these platinum analogues are coming to clinical trials in the United States at the present time in gynecologic malignancies including ovarian carcinoma. Other platinum analogues may also emerge to be of major clinical interest. These analogues offer the distinct hope of less toxic alternatives to cisplatin without a decrease in efficacy.

3.2. Ifosfamide

Ifosfamide (Holoxan, isophosphamide, NSC-10924) is a structural analogue of cyclophosphamide with a similar mechanism of action. Major adverse effects include: dose-limiting urinary tract toxicity manifested primarily as hemorrhagic cystitis, azotemia, and acute renal tubular damage appearing 1 to 2 days after administration of drug and lasting an average 9 days [83]; transient, moderate nausea and vomiting; mild to moderate leukopenia; alopecia; lethargy and confusion; and transient abnormalities of hepatic enzymes. The urinary tract toxicity can be prevented with adequate hydration.

As a part of a broad phase II study of ifosfamide in a variety of neoplasms, 61 patients with advanced ovarian carcinoma and no prior chemo-

Table 11. Results with a number of potentially active new agents in ovarian carcinoma

Drug	Dose and schedule	Response rate
Ifosfamide	300 mg/kg IV over 5 or 10 days	48/61 (79%) (84)
Prednimustine	50 mg/m^2/day p.o. × 28 days	10/36 (28%) (86)
Dihydroxybusulfan	1500 mg/day p.o.	7/26 (27%) (90)
Galactitol	60 mg/m^2 weekly	6/39 (15%) (91)
Mitomycin	10 mg/m^2 IV every 4 weeks	5/38 (13%) (93)
		3/11 (27%) (94)
AMSA	120 mg/m^2 IV every 3 weeks	3/8 (38%) (96)
	120 mg/m^2 IV every 3 weeks	0/9 (0%) (97)
	90 mg/m^2 IV every 3 weeks	2/39 (5%) (98)
	40 mg/m^2/day × 3 days q 3 weeks	1/22 (5%) (99)
	120 mg/m^2 IV every 3 weeks	2/14 (14%) (100)
AZQ	30 mg/m^2 IV every 3 weeks	4/26 (15%) (102)

therapy received either 60 mg/kg/day of ifosfamide intravenously daily for 5 days or 30 mg/kg/day daily for 10 days (Table 11) [84]. Most patients were noted to have received concomitant radiotherapy at low dose to an unspecified area. Furthermore, response criteria were poorly defined. Twenty eight of the 61 patients (46%) achieved a 'full remission', while 20 (33%) demonstrated a 'partial remission'. Despite the confounding factors of concomitant radiotherapy and poor response definition, it would seem that ifosfamide has definite activity against ovarian carcinoma. Whether this agent offers any advantage over cyclophosphamide and other alkylating agents cannot be determined on the basis of available data.

3.3. Prednimustine

Prednimustine (Stereocyt, Leo 1031, NSC-134087) is a chlorambucil ester of prednisolone with a mechanism of action similar to that of chlorambucil. Major adverse effects include: dose-limiting myelosuppression manifested by leukopenia and thrombocytopenia with a prolonged recovery period of up to 6 weeks after drug cessation [85]; transient, mild nausea and vomiting and occasionally a mild cyclical diarrhea; and mild edema.

A single study in advanced ovarian carcinoma has been reported (Table 11). In 36 patients objective responses were observed in 10 (28%) [86]. Among 21 of these patients with no prior chemotherapy, 8 responses (38%) including 2 clinically complete responses were seen, while 2 of 15 (13%) patients with prior chemotherapy with melphalan alone or in combination with adriamycin responded. All patients had previously received radiotherapy. Prednimustine thus appears to have activity similar to that observed with other alkylating agents. Whether this agent offers any advantage over standard alkylating agents is not known.

3.4. Dihydroxybusulfan

Dihydroxybusulfan (Treosulfan), although an alkylating agent structurally similar to busulfan, appears to act by a different mechanism involving the formation of epoxides [87]. The dose-limiting toxicity is myelosuppression similar to that seen with busulfan.

A number of single agent trials of this agent in patients with no prior chemotherapy have been reported [87–90]. All stages of ovarian carcinoma are included in these studies, and response criteria are poorly defined. One of these reports [90] does have sufficient patients with stage III and IV or recurrent disease to permit some conclusions about drug activity. Among 26 patients with advanced or recurrent ovarian carcinoma, 7 (27%) experi-

enced objective regression of disease. The drug thus appears to possess definite anti-tumor activity of an order similar to other alkylating agents. Whether this drug offers any advantages over these other alkylating agents is not clear; no such advantages are apparent from available data.

3.5. Galactitol

Galactitol (dianhydrogalactitol, DAG, NSC-132313) is the alkali conversion product of dibromodulcitol with a mechanism of action similar to other alkylating agents. Adverse effects include dose-limiting myelosuppression and mild nausea and vomiting.

The single study of this drug in ovarian carcinoma, conducted by the Gynecologic Oncology Group [91], utilized a weekly schedule of 60 mg/m^2 given intravenously. Among 39 patients with advanced or recurrent ovarian carcinoma, 2 complete and 4 partial responses were observed for an overall response rate of 15%. All patients had received prior chemotherapy with at least a mustard-type alkylating agent. In view of prior therapy received, the achievement of a 15% response rate suggests that this drug has definite activity of moderate degree in ovarian carcinoma.

3.6. Mitomycin C

Mitomycin C (Mutamycin, NSC-26980) is an antibiotic which acts as an alkylating agent and which is commercially available. The dose-limiting adverse effect is myelosuppression associated with a delayed nadir from 3 to 8 weeks after drug administration and a variable but prolonged recovery period [92]. Both leukopenia and thrombocytopenia are seen and may be severe. Other toxicities include: acute and transient gastrointestinal effects such as nausea, vomiting, anorexia, and stomatitis; a low incidence of mild to moderate azotemia associated with glomerular degeneration but apparently not dose related; alopecia; lethargy, confusion, and fatigue; and severe tissue necrosis at extravasation sites. The recommended dose and schedule are 20 mg/m^2 IV once every 6 weeks. Pharmacologic doses of pyridoxine are usually given with the drug to prevent parethesias which are a manifestation of drug-induced neurotoxicity.

Among 38 evaluable cases with ovarian carcinoma treated with 10 mg/m^2 intravenously every 4 weeks, 5 partial responses were observed (13%) [93]. All patients were refractory to prior therapy with alkylating agents and cisplatinum. This supports the previous report of a series of 11 patients, among whom 3 responders were noted [94]. Mitomycin thus appears to have moderate activity in the treatment of ovarian carcinoma.

3.7. AMSA

AMSA (acridinyl anisidide, NSC-249992) is an acridine derivative thought to act by intercalation between DNA base pairs. The dose-limiting toxicity appears to be myelosuppression manifested by leukopenia and thrombocytopenia with a nadir within 10 days of drug administration followed by rapid recovery [95]. Other adverse effects include: phlebitis at the injection site, cholestasis with increased serum bilirubin, mild to moderate nausea and vomiting, and rashes. Fatal cardiac arrest has also been reported, but its connection with the drug is not firm.

Five studies have been reported on the use of AMSA as a single agent against ovarian carcinoma refractory to prior therapy with at least alkylating agents and cis-platinum [96–100]. These trials were based on the observation of a response in a patient with ovarian carcinoma in a phase I trial of the drug [101]. The overall response rate for all five trials was 9% (8 responses among 92 patients). While this level of activity is modest at best even among patients with extensive prior chemotherapy, one study [96] showed striking correlation between *in vitro* sensitivities and *in vivo* response (100% correlation – 3 of 3 sensitive, 5 of 5 resistant). In any case, the activity of AMSA is not striking but is present.

3.8. AZQ

AZQ (aziridinylbenzoquinone, NSC-182986) is a quinone with an undetermined mechanism of action. The drug's major and dose-limiting adverse effect is myelosuppression. While there are no published data on the use of this agent in ovarian carcinoma, preliminary data from an on-going phase II study of the Gynecologic Oncology Group suggest that the drug may have activity [102]. In 26 patients, all of whom had received prior chemotherapy with at least an alkylating agent and cisplatinum, a schedule of 30 mg/m^2 intravenously every 3 weeks produced 2 complete and 2 partial responses (15%). Although these data suggest moderate drug activity, it should be emphasized that these are results of a preliminary analysis only.

3.9. Summary of active newer agents

These newer agents all appear to possess activity in the treatment of advanced or recurrent ovarian carcinoma. Even in those cases where the level of activity appears to be of a low order, results are significant in that the patients in those trials had received extensive prior therapy with at least an alkylating agent and cisplatin. These drugs thus represent prime candi-

dates for use in future studies of combination chemotherapy in ovarian carcinoma.

4. Cytotoxic drugs of indeterminate value

Certain additional drugs have been reported to induce objective regression of disease in patients with advanced or recurrent ovarian carcinoma (Table 12). The total number treated with each drug is small, and the responses are more anecdotal in nature. The fact that objective response has been reported with these agents, however, makes them of interest for possible further study as single agents in patients who have failed primary therapy.

The first of these agents is a commercially available vinca alkaloid, *vinblastine sulfate* (Velban, vincaleukoblastine), which was noted in a 1976 review of drugs studied in ovarian carcinoma to have induced objective regression in 3 of 20 patients (15%) treated [3]. This drug has more recently been employed as a 5-day continuous infusion in previously treated patients with advanced ovarian carcinoma by the Southwest Oncology Group, but no data are available yet from this on-going trial.

Another vinca alkaloid, *vindesine,* has been used in 3 patients with ovarian carcinoma as a part of a broad phase II trial [103]. At a dose and schedule of 3 mg/m^2 intravenously weekly, one response was observed. This agent thus probably deserves further evaluation in patients with ovarian carcinoma.

Penberol (cis-4-pentoxybenzoyl-bromoacrylic acid) is a bromoacrylic acid derivative and structural analogue of Cytembena which had been previously reported to be an active agent against ovarian carcinoma [104], an activity which could not subsequently be confirmed [105, 106]. Two recent reports from Czechoslovakia from the same investigators, both concerned with an

Table 12. Cytoxic drugs of indeterminate value in ovarian carcinoma

Drug	Patients	Responses
Vinblastine sulfate [3]	20	3
Vindesine [103]	3	1
Penberol [108]	20	11
9-Methoxyellipticine [111]	?	?
Dibromodulcitol [18]	8	1
5-Azacytidine [18]	4	1
4'Epi-doxorubicin [112]	16	1
Indicine-N-oxide [113]	1	1
Macromycin [114]	1	1

ongoing trial of Penberol in advanced or recurrent ovarian carcinoma, suggest a high order of activity for the analogue.

Among the first 10 patients, 70% were reported to have achieved a complete response despite the fact that 8 of these first 10 cases had received prior chemotherapy with multiple agents [107]. In a report on the extension of the same series, the investigators noted 11 complete responses among 20 patients (55%) with remission durations ranging from 8 to 55 months [107]. The dose and schedule employed was 125 mg three times daily by mouth. The major adverse effect noted was diarrhea with tenesmus in approximately one-third of the patients. These results appear promising and would seem to warrant further study of Penberol as a single agent in patients with prior chemotherapy to confirm or deny these observations.

Ellipticine and certain of its derivatives have been noted to be highly cytotoxic substances with an uncertain mechanism of action [109]. While solubility seems to be a major problem for most of these substances, such is not the case for 9-methoxyellipticine (2-methyl-9-hydroxyellipticine). First reported to be active in acute myeloblastic leukemia [110], this compound more recently was evaluated by the EORTC in a variety of solid tumors [111]. Activity was noted in patients with ovarian carcinoma, but the report does not indicate the number of such patients treated, nor the number of responses. It is therefore impossible to draw any conclusions about the possible efficacy of ellipticines in ovarian carcinoma. Further evaluation of these compounds would, however, seem warranted in view of the responses reported and the apparent lack of hematologic toxicity in trials to date.

Five additional drugs have induced objective regressions in patients with ovarian carcinoma. Dibromodulcitol, a precursor of galactitol, was noted to have induced one response in 8 patients treated [18]. Similarly, 5-azacytidine produced one response in 4 patients treated [18]. An analogue of adriamycin, 4′-epi-doxorubicin, was administered at 75 mg/m^2 intravenously every 3 weeks to 16 patients; one response was noted [112]. Indicine-N-oxide, a pyrrolizidine alkaloid, induced an objective regression of less than 50% in a single patient with ovarian carcinoma included in a phase I trial of this agent [112]. Finally, Macromycin, an antitumor antibiotic, was noted to produce a mixed response in one patient with ovarian carcinoma in a phase I trial of this drug [113]. These again represent anecdotal data because of the small number of patients involved, but further study is probably warranted in each instance.

5. Cytotoxic drugs with insignificant activity

A number of additional drugs have been tested for efficacy against ovar-

ian carcinoma. For some of these agents, sufficient data are available to conclude that the compound is of no further interest as a potential treatment for ovarian carcinoma. For other drugs, although no responses have been noted, too few patients have been studied to permit any conclusions to be drawn regarding efficacy.

A total of fourteen drugs fall into the first group, in which sufficient phase II testing has been conducted to demonstrate that, at least at the dose and schedule examined, insufficient activity exists to justify further evaluation in patients with ovarian carcinoma (Table 13) [18, 37–51, 113–125]. The only compounds in this group to induce any responses at all included: dihydroxyanthracenedione (DHAD) [123], which demonstrated 1 response

Table 13. New agents with insignificant activity against ovarian carcinoma

Drug	Dose and schedule	Response rate
ICRF-159 (Razoxane)	2.5 gm/m^2 IV weekly	0/22 (0%) (113)
	750 mg/m^2/day × 3 days q 4 weeks	0/17 (0%) (114)
VP-16-213 (Etoposide)	100 mg/m^2 days 1, 3, 5 q 3 weeks	2/24 (8%) (115)
	60 mg/m^2 days 1–5 q 3 weeks	0/14 (0%) (116)
Spirogermanium	80 mg/m^2 IV every other day	0/16 (0%) (117)
	50 mg/m^2 IV twice weekly	2/18 (11%) (118)
Yoshi	2 mg/kg/day × 5 days q 6 weeks	0/33 (0%) (119)
CCNU	100 mg/m^2 p.o. q 6 weeks	0/31 (0%) (120)
PALA	5 gms/m^2 IV q 3 weeks	0/30 (0%) (121)
Maytansine	1.2 mg/m^2 IV q 3 weeks	0/29 (0%) (122)
DHAD (Dihydroxyanthracenedione)	12 mg/m^2 q 3 weeks	1/26 (4%) (123)
Methyl CCNU	150 mg/m^2 p.o. q 3 weeks	0/26 (0%) (120)
Piperazinedione	9 mg/m^2 IV q 3 weeks	0/26 (0%) (124)
Baker's Antifol	500 mg/m^2 IV weekly	2/25 (8%) (125)
Pyrazofurin	240 mg/m^2 q 4 weeks	0/19 (0%) (116)
Cytembena	200 mg/m^2 twice daily × 5 days q 5 weeks	0/16 (0%) (105)
	200 mg/m^2 twice daily × 5 days q 5 weeks	0/19 (0%) (106)
Vincristine	1.4 mg/m^2 IV weekly	0/17 (0%) (3)

among 26 patients; spirogermanium [118], which produced 2 responses among 34 patients; and Baker's Antifol [125], with which 2 responses among 25 patients were observed. It is true that all patients in these 16 trials had received extensive prior chemotherapy with alkylating agents and, in most instances, cis-platinum. While it may be argued that activity might have been missed as a result, this seems unlikely when one notes that other drugs with known activity do produce responses in more than 10% of similar patient populations [21, 91, 93, 102]. Additionally, it seems unlikely that an altered drug schedule would produce different results. Further interest in these drugs in the treatment of ovarian carcinoma should therefore be limited.

In the second group of drugs, four drugs deserve brief mention. Bleomycin was studied in 12 patients with advanced ovarian carcinoma; no responses were noted [3]. Similarly, DTIC and streptozotocin eacn produced no responses in 9 patients [3]. Finally, another nitrosourea, chlorozotocin produced no responses in 2 patients so treated [126]. While no definite statements can be made concerning the lack of activity on the part of these agents, currently no additional efforts are underway to pursue evaluation of any of the four.

6. Hormonal therapy

Since the ovary is not only a major source of estrogens and progestins but also a target organ for these as well as other hormonal agents, hormonal therapy might well offer a valid approach to the treatment of ovarian carcinoma [127]. Further evidence that such an approach might be valid is found in epidemiologic data which suggest a significant reduction in the incidence of ovarian carcinoma in women using oral contraceptives as compared to age-matched controls [128]. Efforts to define the role of hormonal therapy in the management of ovarian carcinoma include the empirical use of progestins and anti-estrogens in patients with advanced disease as well as more recent studies of estrogen and progesterone receptors in such patients.

6.1. Progestational agents

The use of progestins in the management of advanced ovarian carcinoma was first reported in 1962, when Jolles reported 4 'good' and 1 'fair' response among 10 patients treated with 17-α-hydroxy-progesterone-17-n-caproate [129]. As is the case with many of the early reports, both subjective and objective improvement is reported as response, and the number of

true objective responses by current standards appears to be only 1 out of the 10 patients, a relatively short-lived partial response. Using the same progestin, Varga and Henriksen [130] reported 1 partial responder among 6 patients.

Other progestins have been used with similar results. Ward treated 23 patients with advanced ovarian carcinoma with 17-α-hydroxyl-19-norprogesterone-17-n-caproate [131]. The reported response rate of 65% (15 responses) includes no more than 3 that would be considered objective responses by current standards (all partial responses). Another series using the same agent reported similar results of 1 partial response among 15 patients [132]. Kaufman noted 1 partial response in 11 patients with 17-α-hydroxy-6-methyl-progesterone acetate [133]. Malkasian and associates treated 9 patients with 6-dehydro-6, 17-α-dimethylprogesterone with 2 responders who remained in remission 30 and 45 months later [134]. The most commonly used progestin, however, has been medroxyprogesterone acetate [135–139]. True objective responses with medroxyprogesterone acetate (MPA) have been seen for the most part in less than 10% of cases regardless of route of administration or dose level [135–138]. One exception to this is one recent report [139] of exceptionally high dose therapy (800 mg daily for 30 days followed by 400 mg per day maintenance) which noted 6 complete and 4 partial responders among 23 patients. The partial responses lasted from 4 to 10 months, the complete responses from 5 to 65 months. It should be noted that 8 patients were excluded from analysis because they received less than 3 months of progestin.

In summary, 176 patients with advanced ovarian carcinoma have been treated with progestins. Twenty-two objective responses were observed for an overall response rate of 12% (Table 14). Most responses occurred in patients with serous or endometrioid histology, although relationship of his-

Table 14. Progestin therapy in advanced ovarian carcinoma

Agents	Patients	Responses
17-α-hydroxyprogesterone-17-n-caproate [129]	10	1
[130]	6	1
17-α-hydroxyl-19-norprogesterone-17-n-caproate [131]	23	3
[132]	15	1
17-α-hydroxyl-6-methylprogesterone acetate [133]	11	1
6-dehydro-6, 17-α-dimethylprogesterone [134]	9	2
Megestrol acetate [135]	19	1
[137]	27	1
[138]	25	1
[139]	31	10
Totals	176	22 (12%)

tologic pattern and response is not well defined. With the exception of one series, the objective response rate of advanced ovarian carcinoma to progestins has been minimal. From available data, no conclusions regarding true level of efficacy response duration, optimal choice of progestin, or dose-response relationships can be drawn. The only definite statement that can be made is that progestins do possess a low order of activity in ovarian carcinoma.

6.2. Anti-estrogens

The only other hormonal agents which have been evaluated in the treatment of ovarian carcinoma are the triphenylethylene anti-estrogens (MER-25, clomiphene citrate, nafoxidine, and tamoxifen citrate). The demonstrated efficacy of tamoxifen citrate in breast carcinoma [140] suggests that antiestrogens might be an effective alternative to progestins in ovarian carcinoma. A report of 3 cases of advanced ovarian carcinoma which respond to therapy with anti-estrogens supports this assumption [141]. One patient developed a complete response to nafoxidine, while the other two patients each developed a partial response to tamoxifen citrate.

Additional evidence that antiestrogens have some efficacy in ovarian carcinoma is provided by a series of 13 patients treated with tamoxifen citrate [142]. One partial responder was noted, and an additional 4 patients with rapidly progressive disease stabilized for a period of time. Data on the *in vitro* responsiveness of ovarian carcinoma to tamoxifen citrate also support the efficacy of antiestrogens and suggest that prolonged exposure is needed [143]. Fifteen neoplasms exposed *in vitro* to tamoxifen citrate for 1 hour failed to respond, but 5 of 10 neoplasms exposed continuously for a prolonged period of time responded.

In summary, sparse data (Table 15) suggest that antiestrogens may be active in ovarian carcinoma. The Gynecologic Oncology Group is currently conducting a larger study of tamoxifen citrate in ovarian carcinoma. This trial should provide more definitive answers regarding the role of these agents in ovarian carcinoma.

Table 15. Antiestrogens in ovarian carcinoma

Drug	Patients	Responses
Nafoxidine [141]	1	1
Tamoxifen [141, 142]	15	3
Total	16	4

6.3. Estrogen and progesterone receptors

Estrogen receptors were first noted in a case of ovarian carcinoma in 1975 [144]. A subsequent study of estrogen receptors in a variety of neoplasms included 3 cases of ovarian carcinoma, in one of which a significant titer of estrogen receptors was noted [145].

More recently, several series of patients with ovarian carcinoma report the frequency with which estrogen and progesterone receptors can be found in ovarian carcinoma (Table 16) [146–152]. Assay method and interpretation vary among the series. If authors' criteria are accepted in each case and overall frequency analyzed, one notes that 51% of all cases have evidence of significant titers of both estrogen and progesterone receptors and that 74% have detectable estrogen receptors.

Those lesions positive for both receptors tended to be better differentiated [148–151, 153, 154] and to be associated with longer patient survival and disease-free interval [150, 154]. Whether receptor positivity is associated with specific histologic patterns is not clear with some studies suggesting no correlation [147, 152, 155] and others suggesting that serous carcinomas are associated with higher titers of both receptors [148, 151]. The number of patients is too small to allow definite conclusions to be drawn concerning any of these associations.

Correlation of receptor status with clinical response to hormonal therapy in ovarian carcinoma is based on even fewer cases (Table 17) [141, 142, 152]. Most studies concern themselves with only the frequency with which receptors are found and not the relation between response and receptor levels. It is impossible to determine, on the basis of the 15 reported cases, whether the presence of receptor enhances likelihood of hormonal response.

Table 16. Estrogen and progesterone receptors in ovarian carcinoma

Patients	ER+PR+	ER+PR−	ER−PR+	ER−PR−	References
16	3 (19%)	5 (31%)	0 (0%)	8 (50%)	(146)
21	8 (38%)	7 (33%)	0 (0%)	6 (29%)	[147]
68	52 (76%)	8 (23%)	3 (5%)	5 (7%)	[148]
45	22 (50%)	9 (20%)	0 (0%)	14 (30%)	[149]
21	9 (43%)	4 (19%)	4 (19%)	4 (19%)	[150]
44	17 (39%)	14 (32%)	3 (7%)	10 (22%)	[151]
13	5 (38%)	6 (46%)	1 (8%)	1 (8%)	[152]
228	116 (51%)	53 (23%)	11 (5%)	48 (21%)	

Table 17. Receptor status correlated with clinical response to hormonal therapy in ovarian carcinoma

Receptor status	Patients	Responses	References
ER+PR+	1	1	[141]
ER+PR−	1	0	[152]
ER+PR?	6	0	[142]
ER−PR?	7	1	[142]

6.4. Summary of hormonal therapy

The data cited support the concept that hormonal therapy in the form of progestins or antiestrogens has definite activity in the treatment of ovarian carcinoma. This activity is of a low order and bears no clear-cut relationship to any identifiable characteristic of ovarian carcinomas such as grade or histologic type. While some sources indicate that serous or endometrioid carcinoma or better-differentiated carcinomas are more likely to respond, actual data are insufficient to support these contentions conclusively.

A growing body of information on receptors in ovarian carcinoma suggests that both estrogen and progesterone receptors are found in these lesions in a majority of cases. Whether levels of estrogen and progesterone receptors correlate with response to hormonal therapy remains an unanswered question because of the relatively small number of cases studied.

Because of the low order of activity in unselected cases and the lack of factors which identify those patients more likely to respond, the role of hormonal therapy in the management of patients with ovarian carcinoma is unclear at the present time. Studies of medroxyprogesterone acetate and tamoxifen citrate as single agents in the management of recurrent ovarian carcinoma of known receptor status are currently being conducted by the Gynecologic Oncology Group. The results of these two trials, both of which will involve relatively large numbers of cases, should provide sufficient data to permit conclusions regarding the roles of hormone receptors and hormonal therapy in ovarian carcinoma.

7. Conclusion

Options for effective systemic therapy for ovarian carcinoma include at least 6 established cytotoxic agents (Table 8) plus an additional 8 newer cytotoxic drugs (Tables 10 and 11) as well as two classes of hormonal agents (Tables 14 and 15). That this relative abundance of systemic options is important is underscored by the tendency of ovarian carcinoma to present

at an advanced stage and hence not to be amenable to cure with a surgical approach alone. Good evidence has been cited to indicate that a small percentage of even far advanced patients will achieve long lasting complete responses which may equate to use.

These single-agent data, however, should not be interpreted as suggesting that therapy with single drugs represents the best approach to advanced ovarian carcinoma. Equally as obvious as the efficacy of these drugs is the fact that a vast majority of the patients with advanced disease eventually die of their disease. The use of single agents thus leaves a great deal of room for improvement.

The variety of mechanisms of action cited for the active drugs supports current efforts to develop effective combinations of drugs with high response rates and improved overall survival. Subsequent chapters will describe these efforts in detail. Continued efforts to identify new active agents, however, are warranted in hopes that even more effective single agents can be selected and that these additions to our single-agent armamentation will enhance the development of effective combinations.

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References

1. Kauppila A, Vierikko P, Kivinen S, Stenback F, Vihko R: Clinical significance of estrogen and progestin receptors in ovarian cancer. Obstet Gynecol 61:320–326, 1983.
2. Pollow K, Schmidt-Matthiesen A, Hoffman G, Schweikhart G, Kreinberg R, Manz B, Grill HJ: 3H-estradiol and 3H-R5020 binding in cytosols of normal and neoplastic human ovarian tissue. Int J Cancer 31:603–608, 1983.
3. DeVita VT, Wasserman TH, Young RC, Carter SK: Perspectives on research in gynecologic oncology. Cancer 38:509–525, 1976.
4. Malkasian G, Decker D, Green S, Edmonson J, Jeffries J, Webb M: Treatment of recurrent and metastatic carcinoma of the cervix: Comparison of doxorubicin with a combination of vincristine and 5-fluorouracil. Gynecol Oncol 11:235–239, 1981.
5. Cavin J, Geisler H: Treatment of advanced, unresectable cervical carcinoma already subjected to complete irradiation therapy. Gynecol Oncol 6:256–260, 1978.
6. Brenner F, Forastiere A, Rosenchein N, Jones H, Dillon M, Grumbine F, Tipping S, Aisner J, Burnett L, Greco F, Wiernik P: A Phase II study of spirogermanium in patients with advanced carcinomas of the ovary and cervix. Proc ASCO 1:115, 1982.
7. Thigpen T, Ehrlich C, Blessing J: Phase II trial of maytansine in treatment of advanced or recurrent squamous cell carcinoma of the cervix. A gynecologic oncology group study. Proc ASCO 21:424, 1980.
8. Conroy J, Blessing J, Lewis G, Mangan C, Hutch K, Wilbanks G: Phase II trial of ICRF-159 in treatment of advanced squamous cell carcinoma of the cervix. Proc ASCO 21:423, 1980.

9. Rossof AH, Drukker BH, Talley RW, Torres J, Bonnett J, Brownlee RW: Randomized evaluation of chlorambucil and melphalan in advanced ovarian cancer. Proc AACR-ASCO 17:300, 1976.
10. Piver MS, Barlow JJ, Chung WS: High-dose cyclophosphamide for recurrent or progressive ovarian adenocarcinoma. Cancer Chemother Rep 59:1157–1158, 1975.
11. Hubbard SM, Barkes P, Young RC: Adriamycin therapy for advanced ovarian carcinoma recurrent after chemotherapy. Cancer Treatment Rep 62:1375–1377, 1978.
12. Stanhope CR, Smith JP, Rutledge F: Second trial drugs in ovarian cancer. Gynecol Oncol 5:52–58, 1977.
13. Malkasian GD Jr: Chemotherapy for ovarian cancer. Med Clin North Amer 58:779–792, 1974.
14. Twiggs LB, Morrow CP: Treatment of advanced ovarian carcinoma with high-dose cyclophosphamide after failure on melphalan. Cancer Treatment Rep 61:1369–1371, 1977.
15. Bruckner HW, Ratner LH, Cohen CJ, Wallach R, Kabakow B, Greenspan EM, Holland JF: Combination chemotherapy for ovarian carcinoma with cyclophosphamide, adriamycin, and cis-dichlorodiammineplatinum (11) after failure of initial chemotherapy. Cancer Treatment Rep 62:1021–1023, 1978.
16. Piver MS, Barlow JJ, Yazizi R, Blumenson LE: Melphalan chemotherapy in advanced ovarian carcinoma. Obstetr Gynecol 51:352–356, 1978.
17. Fisher RI, Young RC: Advances in the staging and treatment of ovarian cancer. Cancer 39:967–972, 1977.
18. Thigpen T: The chemotherapy of epithelial ovarian cancer. In: Ballon Sc, ed, Gynecologic Oncology: Controversies in Cancer Treatment, GK Hall, 1981, pp. 272-294.
19. Young RC, Hubbard SH, DeVita VT: The chemotherapy of ovarian carcinoma. Cancer Treat Rev 1:99–110, 1974.
20. Young RC: Chemotherapy of ovarian cancer: past and present. Sem Oncol 2:267–276, 1975.
21. Thigpen JT, Lagasse L, Homesley H, Blessing JA: Cisplatinum in the treatment of advanced or recurrent adenocarcinoma of the ovary: a phase II study of the Gynecologic Oncology Group. Amer J Clin Oncol 6:431–435, 1983.
22. Young RC, Von Hoff DD, Gormley P, Makuch R, Cassidy J, Howser D, Bull JM: Cis-Dichlorodiammineplatinum (11) for the treatment of advanced ovarian cancer. Cancer Treat Rep 63:1539–1544, 1979.
23. Bagley CM, Young RC, Canellos GP, DeVita VT: Treatment of ovarian carcinoma: possibilities for progress. New Eng J Med 287:856–862, 1972.
24. Fisher RI, Young RC: Chemotherapy of ovarian cancer. Surg Clin N Amer 58:143–150, 1978.
25. Taetle R, Dickman PS, Feldman PS: Pulmonary histopathologic changes associated with melphalan therapy. Cancer 42:1239–1245, 1978.
26. Einhorn N: Acute leukemia after chemotherapy. Cancer 44:444–447, 1978.
27. Rosner F: Multiple myeloma and acute leukemia: a review of 104 cases. Proc AACR-ASCO 20:299, 1979.
28. Papavasilion C, Pappas J, Aravantinos D, Kaskarelis D: Treatment of cervical carcinoma with adriamycin combined with methotrexate. Cancer Treat Rep 62:1005–1006, 1979.
29. Young RC *et al.*: Chemotherapy of advanced ovarian carcinoma: a prospective randomized comparison of phenylalanine mustard and high dose cyclophosphamide. Gynecol Oncol 2:489–497, 1974.
30. Alberts DS, Chang SY, Chen HSG, Evans TL, Moon TE: Variability of melphalan absorption in man. Proc AACR-ASCO 19:334, 1978.
31. Brady L, *et al.*: Radiotherapy, chemotherapy and combined therapy in stage III epithelial ovarian cancer. Cancer Clin Trials 2:111–120, 1979.

32. Blom J, Park R, Blessing J: Treatment of Women with disseminated and recurrent ovarian carcinoma with single and multichemotherapeutic agents. Proc AACR-ASCO 19:338, 1978.
33. Omura G, *et al.*: A randomized trial of melphalan versus melphalan plus hexamethylmelamine versus adriamycin plus cyclophosphamide in advanced ovarian adenocarcinoma. Proc AACR-ASCO 20:358, 1979.
34. Smith JP, Rutledge F: Chemotherapy in the treatment of cancer of the ovary. Amer J Obstetr Gynecol 107:691–703, 1970.
35. Wallach RC *et al.*: Thiotepa chemotherapy for ovarian carcinoma: influence of remission and toxicity on survival. Obstetr Gynecol 25:475–478, 1965.
36. Parker RT, Parker CH, Wilbanks GD: Cancer of the ovary: survival studies based upon operative therapy, chemotherapy, and radiotherapy. Amer J Obstetr Gynecol 108:878–888, 1970.
37. Krakoff IH: Nephrotoxicity of cis-dichlorodiammineplatinum (11). Cancer Treat Rep 63:1523–1525, 1979.
38. Von Hoff DD, Schilsky R, Reichert CM, Reddick RL, Rozenceverz M, Young RC, Muggia FM: Toxic effects of cis-dichlorodiammineplatinum (11) in man. Cancer Treat Rep 63:1527–1532, 1979.
39. Wiltshaw E, Kroner T: Phase II of dischlorodiammineplatinum (11) (NSC-119875) in advanced adenocarcinoma of the ovary. Cancer Treat Rep 60:55–60, 1976.
40. Rossof AH, Talley RW, Stephens R, Thigpen T, Samson MK, Groppe C, Eyre HJ, Fisher R: Phase II evaluation of cis-dichlorodiammineplatinum (11) in advanced malignancies of the genitourinary and gynecologic organs. Cancer Treat Rep 63:1557–1564, 1979.
41. Bruckner HW, Cohen CJ, Deppe G *et al.*: Chemotherapy of gynecologic tumors with platinum II. J Clin Hematol Oncol 7:619–632, 1977.
42. Bruckner HW, Cohen CJ, Wallach RC *et al.*: Treatment of advanced ovarian cancer with cis-dichorodiammineplatinum (11): poor-risk patients with intensive prior therapy. Cancer Treat Rep 62:555–558, 1978.
43. Piver MS, Barlow JJ, Lele SB, *et al.*: Cisdichlorodiammineplatinum (11) as third-line chemotherapy in advanced ovarian adenocarcinoma. Cancer Treat Rep 62:559–560, 1978.
44. Wiltshaw E, Subramatian S, Alexopoulos C, Barker GH: Cancer of the ovary: a summary of experience with cisdichlorodiammineplatinum (11) at the Royal Marsden Hospital. Cancer Treat Rep 63:1545–1548, 1979.
45. DiMarco A, Zunino F, Silvestrini R, Gambarricu C, Gambetto RA: Interaction of some daunomycin derivatives with deoxyribonucleic acid and their biological activity. Biochem Pharmacol 20:1323–1328, 1971.
46. Barlow JJ, Piver MS, Chuanz JT *et al.*: Adriamycin and bleomycin alone and in combination in gynecologic cancer. Cancer 32:735–741, 1973.
47. DePalo GM, DeLena M, DiRe F, Luciani L, Valagussa P, Bonadonna G: Melphalan versus adriamycin in the treatment of advanced carcinoma of the ovary. Surg Gynecol Obstetr 141:899–902, 1975.
48. Bonadonna G, Beretta G, Tancini G *et al.*: Adriamycin studies at the Instituto Nazionale Tumori, Milam. Cancer Chemother Rep Part 3, Vol 6:231–245, 1975.
49. Blum RH, Carter SK: Adiamycin: a new anticancer drug with significant clinical activity. Ann Intern Med 80:249, 1974.
50. McGuire WP, Young RC: Ovarian cancer. In Staquet MJ(ed),Randomized Trials in Cancer: a Critical review by Sites. New York, Raven Press, 1978.
51. Worzalla JE, *et al.*: Metabolism of hexamethylmelamine-ring-^{14}C in rats and man. Cancer Res 34:2669–2674, 1974.
52. Takita H, Didolkar MS: Effects of hexamethylmelamine on small cell carcinoma of the lung. Cancer Chemothera Rep 58:371–374, 1974.
53. Wamplu GL, Mellette SJ, Kumperming M, Regelson W: Hexamethylmelamine in the

treatment of advanced cancer. Cancer Chemother Rep 56:505–514, 1972.
54. Wilson WL, Bisel HF, Cole D, Rochlin D, Ramurez G, Madden R: Prolonged low-dosage administration of hexamethylmelamine. Cancer 25:568–570, 1970.
55. Weiss RB: The role of hexamethylmelamine in advanced ovarian carcinoma treatment. Gynecol Oncol 12:141–149, 1981.
56. Wharton JT, Rutledge F, Smith JP, Herson J, Hodge MP: Hexamethylmelamine: an evaluation of its tole in the treatment of ovarian cancer. Amer J Obstetr Gynecol 133:833–844, 1979.
57. Wilson Wl, Schoeder Jm, Bisel HF, Mragek, Hummel RP: Phase II study of hexamethylmelamine. Cancer 23:132–136, 1969.
58. Stanhope CR, Smith JP, Rutledge F: Second trial drugs in ovarian cancer. Gynecol Oncol 5:52–58, 1977.
59. Johnson BL, Fisher RI, Bender RA, DeVita VT, Chabner BA, Young RC: Hexamethylmelamine in alkylating agent resistant ovarian cancer. Cancer 42:2157–2161, 1978.
60. Bonomi PD, Mladineo J, Morris B, Wilbanks G, Slayton RE: Phase II trial of hexamethylmelamine in ovarian carcinoma resistant to alkylating agents. Cancer Treat Rep 63:137–138, 1979.
61. Bolis G, D'Inealci M, Belloni C, Mangioni C: Hexamethylmelamine in ovarian cancer resistant to cyclophosphamide and adriamycin. Cancer Treat Rep 63:1375–1377, 1979.
62. Omura GA, Greco FA, Birch R: Hexamethylmelamine in mustard-resistant ovarian adenocarcinoma. Cancer Treat Rep 65:530–531, 1981.
63. Smith JP, Rutledge F: Random study of hexamethylmelamine, 5-fluorouracil and melphalan in treatment of advanced carcinoma of the ovary. Natl Cancer Instit Monogr 42:169–172, 1975.
64. Ansfield FJ, Schroeder JM, Curreri AR: Five years clinical experience with 5-fluorouracil. JAMA 181:295–299, 1962.
65. Malkasian GD, Decker DG, Mussey E, *et al.*: Observations on gynecologic malignancy treated with 5-fluorouracil. Am J Obstet Gynecol 100:1012–1017, 1968.
66. Vaitkevicius VK, Brennen HJ, Beckett VL, *et al.*: Clinical evaluation of cancer chemotherapy with 5-fluorouracil. Cancer 14:131–135, 1961.
67. Kennedy BJ, Theologides A: The role of 5-fluorouracil in malignant disease. Ann Intern Med 55:719–730, 1961.
68. Young CW, Ellison RR, Sullivan RD, *et al.*: The clinical evaluation of 5-fluorouracil and 5-fluoro-2'-Deoxyuridine in solid tumors in adults. A progress report. Cancer Chemother Rep 6:17–20, 1960.
69. Field JG: 5-Fluorouracil (NSC-19893) treatment of advanced cancer in ambulatory patients. Cancer Chemother Rep 33:45–50, 1963.
70. Hall BE, Good JW: Treatment of far advanced cancer with 5-fluorouracil used alone and in combination with irradiation. Incidence and duration of remission and survival-time data in 223 patients. Cancer Chemother Rep 16:369–375, 1962.
71. Jacobs EM, Reeves WJ, Wood DA, *et al.*: Treatment of cancer with weekly intravenous 5-fluorouracil. Cancer 27:1302–1305, 1971.
72. Sullivan RD, Miller E, Zurek WZ, *et al.*: Re-evaluation of metrexate as an anticancer drug. Surg Gynecol Obstet 125:819–824, 1967.
73. Greenspan EM, Bruckner HW: Comparison of regression induction with thio-tepa or methotrexate in bulky stage IIIB ovarian carcinoma. J Natl Cancer Instit Monogr.
74. Parker LM, Griffiths CT, Yankee RA, Knapp RC, Canellos GP: High-dose methotrexate with leucovorin rescue in ovarian cancer: a phase II study. Cancer Treat Rep 63:275–279, 1979.
75. Omura G, Ehrlich C, Blessing J: A randomized trial of cyclophosphamide plus adriamycin with or without cis-platinum in ovarian carcinoma. Proc ASCO 1:104, 1982.
76. Bradner W, Rose W, Huftalen J: Antitumor activity of platinum analog. Cisplatin: Current

Status and New Developments, Academic Press, New York, 1980, pp 171–182.
77. Wolpert-DeFilippes M: Antitumor activity of cisplatin analogs. In: Cisplatin: Current and New Developments, Academic Press, New York, 1980, pp 183–191.
78. Harrap K, Jones M, Wilkinson R, Clink H, Sparrow S, Mitchley B, Clarke S, Veasey A: Antitumor toxic and biochemical properties of cisplatin and eight other platinum complexes. In Cisplatin: Current Status and New Development, Academic Press, New York, 1980.
79. Calvert AH, Harland SJ, Newell DR, Siddik ZH, Jones AC, McElwain TJ, Raju S, Wiltshaw E, Smith E, Baker JM, Peckham MJ, Harrap KR: Early clinical studies with cis-diammine-1, 1-cyclobutane dicarboxylate platinum II. Cancer Chemother Pharmacol 9:140–147, 1982.
80. Wiltshaw E, Evans BD, Jones AC, Baker JW, Calvert AH: JM8, successor to cisplatin in advanced ovarian carcinoma? Lancet 1:587, 1983.
81. Wiltshaw E, Evans B, Calvert A, Jones A: JM8 alone for previously untreated advanced ovarian carcinoma. Lancet 1:1110, 1983.
82. Creaven P, Mittleman A, Pendyala L, Tseng M, Pontes E, Spurelding M, Moayeir H, Madejeruiaz S: Phase I study of a new anti-neoplastic platinum analogue, cis-dichlorotrans-dihydroxy-bis (isopropyllamine) platinum (IV) (CHIP). Proc ASCO 1:22, 1982.
83. Creavan P, Allen L, Cohen M, Nelson R: Studies on the clinical pharmacology and toxicology of isophosphamide (NSC-109724). Cancer Treat Rep 60:445–449, 1976.
84. Bruhl P, Gunther U, Hoefer-Janker H, Huls W, Scheef W, Vahlensieck, W: Results obtained with fractionated ifosfamide massive-dose treatment in generalized malignant tumors. Int J Clin Pharmacol Biopharm 14:29–39, 1976.
85. Konyves I, Nordenskjold B, Forshell G, Schryver A, Westerberg-Larsson H: Preliminary clinical and absorption studies with prednimustine in patients with mammary carcinoma. Eur J Cancer 11:841–844, 1975.
86. Johnsson JE, Trope C, Mattsson W, Grundsell H, Aspergren K, Konyves I: Phase II study of Leo 1031 (prednimustine) in advanced ovarian carcinoma. Cancer Treat Rep 63:421–424, 1970.
87. Lundvall F: Treosulfan in the treatment of ovarian carcinoma. ACTA Obstet Gynecol Scand 52:Suppl 22, pp 3–11, 1973.
88. Larsen M: Treatment of ovarian carcinoma with treosulfan. ACTA Obstet Gynecol Scand 52:Suppl 22, pp 12–17, 1973.
89. Sorensen H: Ovarian carcinoma treatment with treosulfan. ACTA Obstet Gynecol Scand 52:Suppl 22, pp 18–30, 1973.
90. Fennelly J: Treosulfan (dihydroxybusulphan) in the management of ovarian carcinoma. Br J Obstet Gynecol 84:300–303, 1977.
91. Blom J, Blessing J, Mladineo J, Morgan C, Ehrlich C, Homesley H: Dianhydrogalactitol in the treatment of advanced gynecologic malignancies. Proc AACR-ASCO 21:416, 1980.
92. Crooke ST, Bradner WT: Mitomycin C: a review. Cancer Treat Rev 3:121–139, 1976.
93. Shan M, Catalano R, Creech R: Phase II study of mitomycin in the treatment of advanced ovarian carcinoma. Proc ASCO 2:151, 1983.
94. Baker L, Izbricki R, Vaitkevicius V: Phase II study of porfiromycin versus mitomycin-C utilizing acute intermittent schedules. Med Pediatr Oncol 2:207–213, 1976.
95. Von Hoff DD, Howser D, Gormley P *et al.*: Phase I study of 4'-(9-acridinylamino)-methanesulfon-m-anisidide in B16 melanoma. Pharmacology (Basel) 16:221–225, 1978.
96. Ahmann FR, Meyskens FL, Jr, Moon TE, Durie BG, Salmon SE: *In vitro* chemosensitivities of human tumor stem cells to the phase II drug 4'-(9-acrindinylamino) methanesulfon-m-anisidide and prospective *in vivo* correlations. Cancer Res 42:4494–4498, 1982.
97. Brenner DE, Garbino C, Kasdorf H, Villasanta U, Polcaro R, Yovarone J, Aisner J, Schiffer CA, Wiernik PH: A phase II trial of m-ASMA in the treatment of advanced gynecologic malignancies. AM J Clin Oncol 5:291–295, 1982.

98. Dombernowsky P, Siengenthaler P, Somers R, Hansen HH: Phase II trial of amsacrine (m-AMSA) in advanced ovarian carcinoma. Eur J Clin Oncol 18:71–74, 1982.
99. Hilgers RD, Legha SS, Panettiers FJ, Alberts DS: m-AMSA inepithelial carcinoma of the ovary. A Southwest Oncology Group study. AM J Clin Oncol 6:277–279, 1983.
100. Kearsley JH, Page JP, Levi JA, Woods RL, Tattersall MH, Fox RM, Coates AS: Meta AMSA (m-AMSA) in patients with advanced ovarian carcinoma. Aust NZ J Obstet Gynecol 22:107–109, 1982.
101. Von Hoff DD, Howser D, Gormley P, Bender RA, Glaubiger D, Levine AS, Young RC: Phase I study of methanesulfonamide, N-[4-(9-acrindinylamino)-3-methoxyphenyl]-(m-ASMA) using a single-dose schedule. Cancer Treat Rep 62:1421–1426, 1978.
102. Slayton R: Personal communication.
103. Miller TP, Jones SE, Chester A: Phase II trial of vindesine in the treatment of lymphomas, breast cancer, and other solid timors. Cancer Treat Rep 64:1001–1003, 1980.
104. Skoda V, Jandova A, Novotna J: Comparison of cytostatic activities of cytembena and cyclophosphamide during short term administration to women with gynecological carcinoma. Neoplasm 17:175–181, 1970.
105. Edmonson JH, Decker DG, Malkasian GD, Webb MJ, Jorgensen EO: Phase II evaluation of cytembena (NSC-104801) in patients with advanced ovarian carcinoma resistant to alkylating agents: brief communication. J Natl Cancer Inst 59:1619–1620, 1977.
106. Baker L, Samson M, Izbicki R: Phase II evaluation of Cytembena in disseminated epithelial ovarian cancers and sarcomas. Cancer Treat Rep 60:1389–1391, 1976.
107. Novotna J, Andrysek O: Preliminary results with the new cytostatic Penberol in ovarian carcinoma. Neoplasma 27:77, 1982.
108. Novotna J, Andrysek O, Kobilkova J, Laurova L: Survival of patients with advanced ovarian carcinoma treated with Penberol. Neoplasm 30:245–250, 1983.
109. Dalton L, Demerac S, Elmes B, Loder J, Swan J, Teitei T: Synthesis of the tumor-inhibiting alkaloids ellipticine, 9-methoxy-ellipticine and related pyrido (4,36) carbazoles. Aust J Chem 20:2615–2727, 1967.
110. Mathi G, Hayat M, DeVassas F, Schwarzenberg L, Schneider M, Schlumberger J, Jasmin C, Rosenfield C: Methoxy-9-ellipticine lactate. III. Clinical screening: its action in acute myeloblastic leukemia. Rev Eur Etud Clin Biol 15:541–545, 1970.
111. Paoletti C, LePecq JB, DatXuong N, Juret P, Amiel JL, Rouesse J: Antitumor activity, pharmacology, and toxicity of ellipticines, ellipticinium and 9-hydroxy derivatives: preliminary clinical trials of 2-methyl-9-hydroxy ellipticinium (NSC 264–137). Recent Results Cancer Res 74:107–123, 1980.
112. Trope C: A phase II study of 4′-epi-doxorubicin ovarian carcinoma. Proc ASCO 1:121, 1982.
113. Ohnuma T, Sridhar KS, Ratner LH, Holland JH: Phase I study of indicine N-oxide in patients with advanced cancer. Cancer Treat Rep 66:1509–1515, 1982.
114. Majima H: Exploratory study of macromomycin. Gan To Kagaku Ryoho 9:1568–1573, 1982.
115. Slayton R, Creasman W, Petty W, Bundy B, Blessing J: Phase II trial of VP-16-213 in the treatment of advanced squamous cell carcinoma of the cervix and adenocarcinoma of the ovary: a Gynecologic Oncology Group study. Cancer Treat Rep 63:2089–2092, 1979.
116. Edmonson JH, Decker DG, Malkasian GD, Webb MJ, Jorgensen EO: Phase II evaluation of VP-16-213 (NSC-141540) in patients with advanced ovarian carcinoma resistant to alkylating agents. Gynecol Oncol 6:7–9, 1978.
117. Brenner DE, Jones HW, Rosenshein NB, Forasteiere A, Dillon M, Grumbine F, Tipping S, Burnett L, Grecto FA, Wiernik PH: Phase II evaluation of spirogermanium in advanced ovarian carcinoma. Cancer Treat Rep 67:193–194, 1983.
118. Trope C, Mattson W, Gynning I, *et al.*: Phase II study of spirogermanium in advanced ovarian malignancy. Cancer Treat Rep 65:119–120, 1981.

119. Slavik M, Muss H, Blessing JA, Delgado G: Phase II clinical study of Yoshi 864 in epithelial ovarian carcinoma: a Gynecologic Oncology Group Study. Cancer Treat Rep 66:1775–1777, 1982.
120. Omura G, DiSaia P, Blessing J, Boronow R, Hershchyshyn M, Park R: Chemotherapy for mustard-resistant ovarian adenocarcinoma: a randomized trial of CCNU and methyl-CCNU. Cancer Treat Rep 61:1533–1535, 1977.
121. Muss H, Slavik M, Bundy B, Stehman F, Creasman W: A phase II study of PALA (NSC-224131) in patients with advanced ovarian carcinoma (a Gynecologic Oncology Group study). In press in Amer J Clin Oncol.
122. Thigpen T, Ehrlich C, Creasman W, Curry S, Blessing J: Phase II study of maytansine in the treatment of advanced or recurrent adenocarcinoma of the ovary. Amer J Clin Oncol 6:273–275, 1983.
123. Muss H: Personal communication.
124. Thigpen T, Blessing J, Arseneau J, Homesley H: Phase II trial of piperazinedione (NSC-135758) in the treatment of advanced or recurrent carcinoma of the ovary. In press in Amer J Clin Oncol.
125. Arseneau J, Bundy B, Homesley H, Beecham J: Phase II STUDY OF Baker's Antifolin advanced carcinoma of the ovary. Invest New Drugs 1:185–188, 1983.
126. Gralla RJ, Tan CT, Young CW: Phase I trial of chlorozotocin. Cancer Treat Rep 63:17–20, 1979.
127. Friedman M, Hoffman P, Jones H: Hormone receptor assays in malignant disease. Cancer Treat Rep 5:185–194, 1978.
128. Mishell DR, Jr: Noncontraceptive heath benefits of oral steroidal contraceptives. Am J Obstet Gynecol 142:809–816, 1982.
129. Jolles B: Progestins in the Treatment of advanced malignant tumors of breast, ovary, and uterus. BR J Cancer 16:209, 1962.
130. Varza A, Henriksen E: Effect of 17-hydroxyprogesterone-17-n-caproate on various pelvic malignancies. Obstet Gynecol 23:51, 1964.
131. Ward HW: Progestogen therapy for ovarian carcinoma. J Obstet Gynecol Br Commonw 79:555–559, 1979.
132. Timothy I: Progestogen therapy for ovarian carcinoma. Br J Obstet Gynecol 89:561–563, 1982.
133. Kaufman RJ: Management of advanced ovarian carcinoma. Med Clin North Am 50:845, 1966.
134. Malkasian GD, Jr, Decker DG, Jorgensen EO, Webb MJ: 6-Dehydro-6 17 alpha-dimethyl-progesterone (NSC-123018) for the treatment of metastatic and recurrent ovarian carcinoma. Cancer Chemother Rep 57:241–242, 1973.
135. Malkasian GD, Jr, Decker DG, Jorgensen EO, edmonson JH: Medroxyprogesterone acetate for the treatment of metastatic and recurrent ovarian carcinoma. Cancer Treat Rep 61:913–914, 1977.
136. GalloCurcia C, Casalai A, Gianciotta A, Gionfra T, Rhinaldi M, Terzoli L, Orofino G: Chemo-hormonotherapeutic treatment of 20 cases of metastatic ovarian carcinoma. Clin Ter 90:137–142, 1979.
137. Aabo K, Pedersen AG, Haid I, Dombernowsky P: High-dose medroxyprogesterone acetate (MPA) in advanced chemotherapy-resistant ovarian carcinoma: a phase II study. Cancer Treat Rep 66:407–408, 1982.
138. Trope C, Johnsson JE, Sigurdsson K, Simonsen E: High-dose medroxyprogesterone acetate for the treatment of advanced ovarian carcinoma. Cancer Treat Rep 66:1441–1443, 1982.
139. Geisler HE: Megestrol acetate for the palliation of advanced ovarian carcinoma. Obstet Gynecol 61:95–98, 1983.
140. Taznon H: Antiestrogens in treatment of breast cancer. Cancer 39:2959, 1977.

141. Myers M, Moore GE, Major FJ: Advanced ovarian carcinoma: response to antiestrogen therapy. Cancer 48:2368–2370, 1981.
142. Schwartz P, Kenting G, Maclusky N, Naftolin F, Eisenfeld A: Tamoxifen therapy for advanced ovarian cancer. Obstet Gynecol 59:583–588, 1982.
143. Lazlo J, Schwartz P, Maclusky N, Eisenfeld A: *In vitro* responsiveness of ovarian epithelial carcinomato tamoxifen. Proc ASCO 2:153, 1983.
144. Golumb HM, Thompson S: Estrogen receptor. Therapeutic guide in an undifferentiated metastatic carcinoma in women. Arch Intern Med 135:942, 1975.
145. Martin JD, Hahnel R: Estrogen receptor studies in carcinoma of the endometrium, carcinoma of the uterine cervix and other gynecological malignancies. Aust NZ J Obstet Gynecol 18:55–59, 1978.
146. Holt J, Caputo T, Kelley K, *et al.*: Estrogen and progesterone binding in cytosols of ovarian adenocarcinoma. Obstet Gynecol 53:50–58, 1979.
147. Janne O, Kauppila A, Syrjala P, Vihko R: Comparison of cytosol estrogen and progestin receptor status in malignant and benign tumors and tumor-like lesions of human ovary. Int J Cancer 25:175–179, 1980.
148. Kauppila A, Vierikko P, Kivinen S, Stenback F, Vihko R: Clinical significance of estrogen and progestin receptors in ovarian cancer. Obstet Gynecol 61:320–326, 1983.
149. Jones L, Jolles C, Edwards C, Freedman R, Gallager H: Estrogen and progesterone receptors in advanced ovarian carcinoma. Proc AACR 24:176, 1983.
150. Leibach S, Miller N, Slayton R, Graha J, Miller A, Yordan E, Wilbanks G, Dunne C: Hormone receptors in ovarian carcinoma. Proc AACR 24:176, 1983.
151. Pollow K, Schmidt-Matthiesen A, Hoffman G, Schweikhart G, Kreinberg R, Manz B, Grill HJ: 3H-estradiol and 3H-R5020 binding in cytosols of normal and neoplastic human ovarian tissue.
152. Jolles CJ, FriFreidman RS, Jones LA: Estrogen and progestogen therapy in advanced ovarian cancer: preliminary report. Gynecol Oncol 16:352–359, 1983.
153. Lele SB, Piver MS, Barlow JJ, Chatterjee S, Schneider S, Ip M: Comparison of cytosol estrogen receptor status in ovarian carcinoma using different radiolabeled ligands and methods. J Surg Oncol 21:155–158, 1982.
154. Creasman WT, Sasso RA, Weed JC, Jr, McCarty KS, Jr: Ovarian carcinoma: histologic and clinical correlation of cytoplasmic estrogen and progesterone binding. Gynecol Oncol 12:319–327, 1981.
155. Freidman M, Lazlos M, Markowitz A, Jones H, Resser K, Hoffman P: Estradiol and progesterone receptors in ovarian cancer-clinical and pathological correlation. Clin Res 27:385A, 1979.

8. Multiagent chemotherapy in advanced ovarian carcinoma

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Introduction

Epithelial ovarian carcinoma is unique among pelvic malignancies and, in fact, among adult adenocarcinomas, for its responsiveness to therapeutic interventions. Using all three commonly employed cancer treatment strategies, surgery, radiation therapy, and chemotherapy, ovarian tumors may be debulked and palpable masses reduced. With such success at reducing tumor volume, one would suspect that major strides in improving survival in this tumor have taken place. In fact, dealing with this tumor remains a frustrating experience to all cancer specialists. Advanced ovarian carcinoma (FIGO Stages III and IV) remains the most common gynecologic cancer killer with 18,200 estimated new cases and 11,500 estimated deaths in 1983 (6% of all female cancer deaths) [1].

Until recently no therapeutic maneuver beyond the initial treatment, chemotherapy with single agents or irradiation, have been able to alter the rapid downhill course. In 1976, DeVita *et al.* [2] identified a number of major problems which have inhibited progress in the treatment of ovarian carcinoma. These included: 1) the inability to diagnose and detect occult tumor in asymptomatic women in the post-operative period, 2) chronic understaging with failure to apply therapy at the appropriate point in the natural history of the disease, 3) ignorance concerning selection of proper therapy for individual patients based upon the absence of controlled clinical trials, and 4) the lack of access to patients by medical oncologists, the major individuals developing and identifying new drugs. The result was slow identification of additional clinical tools beyond radiation therapy and largely ineffective chemotherapy.

Since DeVita's publication in 1976, major changes in the approach to treatment of patients with ovarian carcinoma have occurred. Multidisciplinary treatment teams at major centers have brought together major cancer subspecialists. Many large, prospective randomized trials have identified

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single agents and, now, multiagent combinations which may, in fact, change the natural history of this disease in selected patients. New drugs, particularly cisplatin, have been identified and incorporated in the early postoperative treatment for patients with advanced stage ovarian carcinoma. We believe that multiagent chemotherapy incorporating cisplatin will be able to cure a small percentage of these patients in the near future and may provide extended palliation in the form of durable (greater than 6 months) responses in a large segment of this patient population. New antineoplastic agents and therapeutic approaches are on the horizon which promise further improvement in survival for ovarian carcinoma patients.

In this chapter, we shall review recently published multiagent chemotherapy trials for patients with advanced ovarian carcinoma. Chronological developments will reveal a new 'state of the art' over the last 5 years which is rapidly changing as new agents and concepts are evaluated and published.

Rationale for multiagent chemotherapy

Skipper *et al.* [3] first demonstrated a synergistic additive therapeutic effect of two antineoplastic mechanisms, e.g. an alkylating agent and an antimetabolite. The use of different mechanisms of drug action such as alkylation and intercalation, combined with overlapping toxicity has led to the successful use of multiagent chemotherapy in a variety of neoplasms – Hodgkins disease, nonHodgkins lymphomas, Wilms tumors, acute lymphocytic leukemia – in children [4] to name a few.

A number of single agents have important antitumor activity against epithelial ovarian carcinoma. They include a variety of alkylating agents (e.g. thiotepa, cyclophosphamide, and chlorambucil) [5–8], cisplatin [9–11], hexamethylmelamine [12, 13], and adriamycin [14–16]. Hexamethylmelamine is noncross-resistant with other alkylating agents [17]. These agents have formed the nucleus of combination regimens to be tested in ovarian carcinoma.

Difficulties in assessing data from multiagent chemotherapy trials

Balancing prognostic variables

Therapeutic efficacy from any treatment program may be defined by the number or percentage of patients responding to treatment, by the length of disease or relapse free interval after achieving a response, and ultimately, by the overall survival. In order to determine whether any new therapy is ben-

eficial it must be compared to 'standard' treatment. In the case of ovarian carcinoma, this becomes a difficult task. Multiple pretreatment variables effect response to chemotherapy and survival. In order to accurately compare treatments for ovarian carcinoma, a study should not only be randomized but also be balanced for pretreatment variables.

The variable of greatest importance is the amount of residual tumor left at initial laparotomy. Patients with residual tumor less than 1.5 to 2.0 cm have improved survival to any initial treatment regimen when compared to those with larger residual tumor masses [18–21]. The size of regional tumor bulk has considerable influence on the chances of longer term survival after chemotherapy.

The degree of tumor differentiation (grade) has some prognostic importance, although the relative importance of this variable is not as well defined for all types of treatment regimens. Generally, patients with well differentiated tumors tend to have longer survivals while the contrary is true for patients with poorly differentiated tumors [22–24]. Prior exposure to chemotherapy [25, 26] or radiation therapy are important factors for both response and survival. Exposure to either modality, single agents or multiagent chemotherapy markedly reduces response rates to a subsequent multiagent regimen. Previously treated patients are nortoriously resistant to further therapy of any kind and usually have limited (<6 months) survivals [26].

Age appears to be an important prognostic factor. The elderly do not respond to chemotherapy as well and do not survive as long. This may be due, in part to their inability to tolerate the toxicity of aggressive chemotherapy. Subsequent dose reductions result in the loss of therapeutic efficacy. Finally, the performance status, which represents the general health of the patients and, perhaps, the cellular tumor burden, is an important prognostic factor.

With all of the above described variables, it is no wonder that individual non-randomized studies are difficult to interpret. Different treatment populations, surgical technique, and intentional and unintentional patient selection contribute to the confusion. While the randomized study is an improvement over the single arm study, the difficulty in balancing treatment arms is an almost impossible task without the very large patient resource available only to cooperative groups.

Defining a response

Advanced ovarian carcinoma is a classic hidden abdominal tumor and does not lend itself to clinical evaluation. Criteria of response are not uniform and a wide variety of definitions exist. The most commonly used

definition used is that of 'clinically evaluable response'. A 'clinical evaluation' in one series may be the physical examination of palpable tumor while in another, it may include a variety of sophisticated diagnostic tests such as ultrasound and computed tomography. Even these diagnostic tests have marked limitations in the detection of small volume (<2 cm) residual disease; particularly in isodense locations such as peritoneum, omentum, and mesentery [27, 28]. Therefore, the 'complete clinical response' so commonly used could include patients who pathologically may be disease free to patients with large masses which are not detectable by physical examination or noninvasive radiologic tests. Such variables can have major implications upon the interpretation of 'response' in randomized studies. Because of such problems, survival becomes the ultimate criterion of treatment efficacy. Unless many prognostic variables are balanced, the randomized study has less meaning. Again, only large, cooperative group studies can obtain a large enough sample to balance the many variables.

The keystone of initial evaluation is the laparotomy and the surgeon has the major responsibility in determining the extent of intraabdominal tumor. Since many patients have maximal tumor resection at primary surgery, fewer patients will have clinically palpable or radiologically detectable tumor to follow. Second look laparotomy is the only way to definitively assess treatment results and, with the exception of survival, is the best way to evaluate the results of randomized treatments [29–31].

Combination chemotherapy regimens that exclude cisplatin

Early multiagent studies

In 1962, Greenspan evaluated the effects of administering methotrexate with ThioTepa in 35 patients with advanced ovarian carcinoma [40]. ThioTepa was given at a dose of 60 mg daily for 4 days and methotrexate at a dose of 5 to 12.5 mg daily until stomatitis occurred. The combination 'induced strikingly rapid and relatively sustained major regressions of massive abdominal tumors among two-thirds of 23 fresh Stage IV cases. The overall response rate of 70% appeared slightly better than the usual 40 to 50% level expected from ThioTepa, chlorambucil or Cytoxan alone'. A subsequent report [32] of 96 evaluable patients on the same regimen yielded a response rate of 67% and a median survival of 17.4+ months (Table 1). Interestingly, Greenspan, in discussing his data [32], pointed out that other alkylating trials had achieved similar response rates and durations of response. He noted the need for critical analysis of medical risk factors, and suggested the need for a controlled, randomized study.

Smith and Rutledge [33] published a series of 47 patients (Table 1),

Table 1. Non-randomized combination chemotherapy studies without cisplatin

Author	Yr	Drugs	*N* =	Response (%)	CR (%)	Median DFI (months)		Median survival (months)		Comments
						CR	PR	CR	PR	
Greenspan [32]	1968	TTP+MTX	96	60 (67)	–	ALL 10.5		ALL 17.4+		All palpable disease, no surgical confirmation, first major trial to evaluate drug combination, MTX given orally to toxicity
Smith [33]	1970	CTX+5FU ACT-D	47*	18 (38)	4 (9)	–	–	–	–	
Lokich [34]	1972	5–FU+MTX+ VCR+CTX+PRED	2	2	1					Many tumors studied, apparently active in ovarian carcinoma
Parker [35]	1980	ADR+CTX	41 12*	34 (83) 2 (17)	20 (49)	9	7	16	11.5	Doses were escalated to toxicity, 12 surgically proven CR's
Neijt [36]	1980	MTX+5–FU+ CTX+HEX	23 14*	12 (52) 3 (21)	5 (22) 1 (7)	ALL 8+		–	–	Large residual tumor bulk with a 9/23 Stage IV patients, short follow-up, all responses documented by laparoscopy or laparotomy
DePalo [37]	1981	HEX+CTX+ MTX+5–FU	31	13 (42)	7 (23)	30	9.5	21+	–	States results inferior to NCI (Young, 46) due to larger number of PR, only 4 pts surgically restaged
Fiorentino [38]	1982	ADR+CTX	70	36 (51)	0	–	–	–	–	Short follow-up (median 8 mos)
Jobson [39]	1983	CTX+ADR+ ARA–C	17 9*	3 (18) 4 (44)	3 (18) 3 (34)	12 8	– 6	– –	– –	Short follow-up, longer survival with less tumor burden at start

CR = Complete response, PR = Partial response, DFI = Disease Free Interval, TTP = ThioTepa; MTX = methotrexate, ADR = adriamycin, CTX = cyclophosphamide, 5-FU = 5-fluorouracil, HEX = hexamethylamelamine, ARA-C = cytosine arabinoside, ACT-D = actinomycin-D, PRED = prednisone

* Previously treated with chemotherapy or radiation therapy.

Table 2. Randomized combination chemotherapy studies without cisplatin

Author	Yr	Drugs	Evaluable pts	Response (%)	CR (%)	Median DFI (months) CR	Median DFI (months) PR	Median survival (months) CR	Median survival (months) PR	Comments
Smith [41]	1972	LPAM	50	21 (42)	10 (20	–	–	17% 2 yr survival		No difference between treatment arms
		CTX + 5FU	47	21 (45)	14 (30)	–	–	17% 2 yr survival		
Barlow [42]	1976	HDMTX	13	2 (15)	0	–	10,2	–	–	All patients alkylating agent failures, claims 2 drugs superior
		CTX + HDMTX	16	7 (44)	0	–	2	–	–	
Barlow [43]	1977	LPAM	49	17 (35)	9 (18)	–	–	–	–	Combination felt superior based upon statistically improved progression free interval and response rate
		ACT-D + 5FU + CTX	49	26 (53)	14 (29)	–	–	–	–	
DePalo [44]	1977	ADR	12	3 (25)	–	–	–	–	–	No difference in response rates
		ADR + LPAM	14	3 (22)	–	–	–	–	–	
Bradovsky [45]	1977	LPAM	114	30 (26)	19 (17)	–	–	28	–	No difference in survival
		CTX + MTX + 5FU	110	45 (40)	21 (19)	–	–	17	–	
Young [46]	1978	LPAM	37	20 (54)	13 (33)	ALL – 25		ALL – 17		Statistically improved response and survival for combination in grade 2 and 3; 2 cm residual tumor at initial laparotomy
		HEX + CTX + MTX + 5FU	40	30 (75)	17 (43)	ALL – 30 +		ALL – 29		
Bruckner [47]	1979	LPAM	70	8 (11)	4 (6)	–	–	–	–	Preliminary data, suggested early advantage for adriamycin arm
		TTP + MTX	72	11 (15)	4 (6)	–	–	–	–	
		CTX + ADR + 5FU	71	21 (30)	7 (10)	–	–	–	–	
		TTP Alternating with CTX + ADR + 5FU	62	13 (21)	2 (3)	–	–	–	–	
Klaassen [48]	1979	LPAM + 5FU + HDMTX	56	21 (38)	12 (21)	10	8	ALL 14		No difference before treatment arms, 5FU and MTX in sequence after LPAM failure were inactive
		LPAM (followed by 5FU and MTX in sequence)	52	24 (46)	12 (23)	8	4	ALL 14		

LPAM = L-phenylalanine mustard (melphalan), HEX = hexamethylmelamine, CTX = cyclophosphamide, MTX = methotrexate, 5FU = 5-fluorouracil, ACT-D = actinomycin-D, TTP = thiotepa, HDMTX = high dose methotrexate; CR = complete response; PR = partial response, DFI = disease free interval.

Table 2. (continued)

Author	Yr	Drugs	Evaluable pts	Response (%)	CR (%)	Median DFI (months) CR	Median DFI (months) PR	Median survival (months) CR	Median survival (months) PR	Comments
Edmonson [49]	1979	CTX	35	11 (31)			—	ALL – 12		No difference in response or survival rates
		CTX + ADR	36	13 (36)			—	ALL – 12		
Park [50]	1980	LPAM	61	18 (30)	12 (20)	ALL – 5.5				No difference among treatment arms
		LPAM + 5FU	48	12 (25)	8 (17)	ALL – 7.6				
		LPAM + 5FU + ACT – D	48	15 (31)	8 (17)	ALL – 6.0				
		CTX + 5FU + ACT-D	21	7 (33)	2 (10)	ALL – 2.8				
Barlow [51]	1980	CTX + HDMTX	21	14 (67)	10 (48)	15+	—	ALL – 14+		Claims improvement with CTX-HDMTX
		CTX + 5FU	23	7 (32)	3 (14)	15+	—	ALL – 15+		
Turbow [52]	1980	LPAM	23	7 (30)	1 (3)	—	—	ALL – 14		No difference in survival
		CTX + ADR	24	17 (71)	2 (8)	—	—	ALL – 14		
Schwartz [53]	1981	CTX + ADR	17	10 (59)	6 (35)	—	—	ALL – 16.7		No difference between treatment arms
		CTX + HEX	20	10 (50)	6 (30)	—	—	ALL – 13.7		
Medical Research Council (United Kingdom) [54]	1981	CTX	131	29 (22)	—	—	—	ALL – 10		No difference between treatment arms
		CTX + HEX + MTX	130	43 (33)	—	—	—	ALL – 12		
Tropé [55]	1981	LPAM	72	29 (40)	7 (10)	8.1	5.5	16	14.5	Combination arm statistically better for response, median duration of response, and median survival
		LPAM + ADR	70	44 (63)	21 (30)	23+	7.0	24+	15.2	
Omura [56]	1983	LPAM	96	24 (38)	13 (20)	ALL – 5.0		ALL – 12.3		Poor prognosis patients, no survival difference among the arms
		LPAM + HEX	154	50 (52)	27 (28)	ALL – 6.3		ALL – 14.2		
		CTX + ADR	119	37 (47)	23 (32)	ALL – 6.6		ALL – 13.5		

LPAM = L-phenylalanine mustard (melphalan), HEX = hexamethylmelamine, CTX = cyclophosphamide, MTX = methotrexate, 5FU = 5-fluorouracil, ACT-D = actinomycin-D, TTP = thiotepa, HDMTX = high dose methotrexate; CR = complete response; PR = partial response, DFI = disease free interval.

refractory to L-phenylalanine mustard (LPAM) (Melphalan), who were treated with a three drug combination including cyclophosphamide, actinomycin-D, and 5-fluorouracil. The overall response rate of this group was 38% with 4 (9%) complete responders. No survival data were reported.

Both of the above groups compared their combinations in randomized studies to standard single agent alkylating agents. Neither combination was superior to single agent therapy (Table 2, Bruckner, 1978 and Smith, 1972) [41, 47]. Confirmation of these data has been published by the Gynecologic Oncology Group (GOG) [50] (Table 2, Park, 1980). The multiarm design of the GOG study compared 2 and 3 drug combinations to standard single alkylating agents and found no advantage for any multiagent arm. Barlow and Piver [43] (Table 2, Barlow, 1977) maintained that the 3 drug combination (actinomycin-D, 5-fluorouracil, and cyclophosphamide) provides a statistically higher response rate and a lower progression rate than LPAM alone. Unfortunately, it is not possible to detemrine whether the combination provides a survival advantage.

Alkylating agents and methotrexate

The combination of methotrexate and an alkylating agent continued to receive attention, in spite of the minimal activity of methotrexate as a single agent in ovarian carcinoma [57]. Barlow and Piver [42] have recommended high dose methotrexate and cyclophosphamide based upon data in previously treated patients (Table 2, Barlow, 1976) and in previously untreated patients (Table 2, Barlow, 1980) [51]. In the former study, responses were extremely short and no statistical data were presented to confirm the superiority of the combination. In the latter study, in which the response rate for the methotrexate arm is significantly better than the 5-fluorouracil arm, survival information given suggests no difference between the two treatments. The randomization in this study to *C-Parvum* further complicates the study and makes any comparison with historical, single agent alkylator therapy data invalid. Based upon other randomized data (Table 2, Bruckner, 1979) [47] the two drug combination of an alkylating agent and methotrexate offers no advantage over single drug treatment.

Alkylating agent and hexamethylmelamine

Randomized studies comparing the combination of an alkylating agent and hexamethylmelamine to other combinations or to single drug chemotherapy (Table 2, Schwartz 1981, Omura 1983) [53, 56] have not shown superiority for this combination. The discrepancy between the studies of

Schwartz *et al.* [53] and Omura *et al.* [56] for an alkylating agent and hexamethylmelamine may relate to the lower pretreatment residual tumor in the former study. The overall survival of patients in both studies are similar.

Alkylating agent and adriamycin

Cyclophosphamide and adriamycin have significant first line activity [35]. This combination is of interest because 1) both adriamycin and cyclophosphamide alone have significant antitumor activity in ovarian carcinoma, 2) these two drugs have demonstrated synergism in animal tumor [58] and in other human tumors, particularly breast carcinoma and non-small cell lung cancer [60, 61], and 3) in the study of Parker et al. [35] the drug doses were escalated to toxicity. In spite of the high response rate (83%), the disease free interval and median survival in this study [35] were not appreciably different from single alkylating agent data. A high proportion of patients obtained complete clinical responses (20 of 34), 12 of which were pathologically documented by laparotomy or laparoscopy with washings. Patients with pathologically documented complete responses had prolonged survivals (12 to 60+ months). However, the median survival for all complete responders was similar to those obtained with single agent alkylating agents. When this combination was tested in a randomized study by the GOG (Table 2, Omura, 1983) no differences in response and survival were found. The randomized study of Tropé supports Parker's conclusions and attributes the improved survival in the LPAM-adriamycin arm to increased complete responses and resultant long term survivals [55]. Other randomized studies (DePalo, 1977, Edmonson, 1979, Tubow, 1982) [44, 49, 52] fail to show improvements with the adriamycin combination.

A number of important differences exist between the studies of Parker *et al.* [35] and the GOG [56]. First, the patients treated on the GOG study all had 3 cm residual disease at the beginning of treatment, whereas in the data of Parker *et al.*, over 25% of patients had residual tumor bulk of less than or equal to 1.5 cm and another third had significant disease resected, although to an unknown extent. Second, all patients in the GOG study received 50 mg/m^2 of adriamycin and 500 mg/m^2 of cyclophosphamide whereas approximately 25% of patients in the study of Parker *et al.* received doses of adriamycin ranging from 60–100 mg/m^2 and cyclophosphamide 750–2000 mg/m^2. Finally, 12 of 20 complete responders in the study of Parker *et al.* were pathologically documented by laparoscopy and/or laparotomy. Differences in these two studies suggest that the combination of cyclophosphamide at higher doses, particularly in patients whose tumor is adequately resected, may indeed improve upon single agent-alkylator therapy primarily

because of an increase in patients with complete pathologic responses who are long term survivors. Because the study of Parker *et al.* was not randomized, we do not know whether meticulous surgery and aggressive, high dose single agent-alkylator chemotherapy postoperatively may produce the same results. Data from the National Cancer Institute [46] suggest this indeed may be the case. An aggresive alkylating agent – adriamycin combination warrants further investigation in controlled trials in patients with minimal residual tumor against a cisplatin based combination. The GOG has recently completed such a study (Table 5, Omura, 1982) [111].

Could a dose-response relationship in patients with minimal residual disease account for the high pathologic complete response rates seen in the study of Parker *et al.* [35]? A prospective study from the National Cancer Institute [62] comparing standard, oral dose LPAM to high dose cyclophosphamide revealed no advantage to the high dose schedule. Further data evaluating dose response relationships to other single agents are lacking. Initial data with higher dose cisplatin given over 5 days [114] does not suggest an advantage for the high dose cisplatin in combination, but the daily times five scheduling may be a problem with this study. Further work to answer this question is warranted.

Hexa CAF versus LPAM

The National Cancer Institute study comparing HEXA-CAF to melphalan (Table 2, Young 1978) [46] is regarded as major support for the superiority of combination chemotherapy over single agent treatment based upon improved response rates and survival for the combination group. This study has been criticized because patients were a median of 10 years younger than those in most series (i.e. a better prognostic group), the response rates of the melphalan control group, particularly the number of complete responders, were higher than those usually achieved by others and by the long period of time (18 months) required until a survival advantage was observed [91]. In addition, the survival advantage for the combination arm was found only in those patients with moderately well differentiated (Broders grades 2 and 3) tumors. Furthermore, the methods of data analysis in this study have also been challenged [51, 54].

We suspect that the high complete response rates in the LPAM arm and prolonged survivals are due to the higher proportion of patients treated who had minimal residual tumor, and, perhaps, the more aggressive primary diagnostic techniques [19, 20] which 'upstaged' patients to higher (i.e. more patients in stage III) stage than would normally be found in studies from other institutions or cooperative groups. This would result in a higher proportion of 'good prognosis' stage III patients being treated and a higher complete response rate. In spite of these shortcomings, this study shows that

improved survival, most likely due to the higher complete pathologic responses with long term remissions, can be obtained in a significantly higher percentage of patients receiving multiagent chemotherapy than in those receiving single agent treatment. Therefore, a rationale and precedent was set for further multiagent chemotherapy trials in ovarian carcinoma.

Combined chemotherapy regimens which include cisplatin in previously treated patients

Cisplatin is clearly an active agent in ovarian cancer. An optimal dose has not been determined and most investigators administer the drug as a single infusion over 2 to 3 hours at a dose of 50 mg/m^2 to 100 mg/m^2. Cisplatin has been incorporated into many combination chemotherapy combinations. The initial studies incorporated cisplatin into combinations in previously treated patients. Data from these studies are tabulated in Table 3. Of importance are the consistent responses obtained, ranging from 14% to 72%. Most investigators report a small percentage of clinical complete response. The durations of response, complete or partial, are short (medians of 4–13.5 months) and few long term survivals are documented.

The addition of cisplatin in the treatment of patients resistant to alkylating agents has produced important changes. Previously, Stanhope *et al.* [26] reported a 6.1% complete plus partial response rate to second trials of chemotherapy in 360 patients with advanced ovarian carcinoma treated at M.D. Anderson Hospital. This observation is supported by the failure of sequential single agent treatment regimens to improve upon the response rates and survival of first line (usually alkylating agent) treatment (Table 2, Klaassen, 1979) [48]. Piver *et al.* observed that a cisplatin-based regimen given to patients resistant to multiple agents (rather than single alkylating agents) is ineffective (Table 3, Piver, 1981) [68].

Because of the availability of single tumor cells from pleural or ascitic fluids, the *in vitro* chemosensitivity of human ovarian carcinoma in the human tumor clonogenic assay [79, 80] has been published. These studies suggest high sensitivities of ovarian tumor cells to vinblastine as well as to cisplatin. The synergistic cytotoxic effects of the vinblastine-cisplatin and vinblastine-bleomycin combinations, documented using the human tumor clonogenic assay data, have been clinically evaluated by Ehrlich *et al.* (Table 3) [73] and by Surwit *et al.* (Table 3) [78]. While the numbers of treated patients are small, the response rates, including numbers of complete responders, suggest that a cisplatin-vinblastine based combination may be useful in first line treatment. The multiple studies of various cisplatin based treatment combinations verify the improved tumor responses and survivals in this group of patients who are generally resistant to further therapeutic attempts.

Table 3. Cisplatin containing chemotherapy in patients with alkylating agent resistant ovarian carcinoma

Author	Yr	Drugs	Evaluable pts	Response (%)	CR (%)	Median DFI (months)		Median survival (months)			Comments
						CR	PR	CR	PR	NR	
Vogl (ECOG) [63]	1979	DDP+ADR+ HEX	27	18 (67)	5 (19)	6+	10+	–	–	–	Toxicity of regimen tolerable
Alberts (SWOG) [64]	1979	DDP+HEX+ ADR+5FU	29	14 (48)	2 (7)	–	–	–	–	–	
Alberts (SWOG) [64]	1979	DDP+HEX+ 5FU	74	23 (31)	0	–	4.3	–	14	–	Prior treatment with CTX+ADR
Kane [65]	1979	DDP+CTX+ HEX+ADR	35	17 (49)	7 (20)	9+	4	–	–	–	
Vogl [67] (Einstein)	1980	DDP+HEX+ ADR	49	26 (52)	5 (10)	6	6	11.5	–	5	
Wallach	1980	DDP+ADR	38	14 (37)	7 (18)	–	–	20.3	11.9	–	DDP an important addition to ADR
Piver [68]	1981	DDP+CTX+ ADR+HEX+	20	0	0	–		–	–	–	All pts with a progressive disease after other multiagent regimens, too toxic
Bruckner [69]	1981	DDP+CTX+ ADR+HEX	21	10 (49)	3 (14)	–	–	Median – 15			
Vogl [70]	1982	DDP+HEX	38	21 (55)	9 (24)	9	6.5	10.3	3.8	–	
Sessa [71]	1982	DDP+CTX+ MTX+5FU	35	5 (14)	0	–	10.5	–	14	–	Too toxic after progression on multiagent regimen

DDP = cisplatin, HEX = hexamethylmelamine, ADR = adriamycin, CTX = cyclophosphamide, 5FU = 5-fluorouracil, VBL = vinblastine, BLEO = bleomycin, MTX = methotrexate; CR = complete response, PR = partial response, NR = no response, DFI = disease free interval.

Table 3. (continued)

Author	Yr	Drugs	Evaluable pts	Response (%)	CR (%)	Median DFI (months)		Median survival (months)			Comments
						CR	PR	CR	PR	NR	
Bruckner [72]	1982	DDP+CTX+ ADR+HEX	18	13 (72)	6 (33)	–		75% alive at 13 mos			11 patients progressive on DDP or DDP combination
Neijt [73]	1982	DDP+ADR	20	5 (25)	–	ALL–5.3		ALL–6.9			2nd line treatment ineffective and toxic
		DDP+ADR+ HEX	21	4 (19)	–	ALL–4.1		ALL–5.3			
Bernath [74]	1982	DDP+ADR+ HEX	27	11 (41)	6 (22)	–	–	ALL–7.5			Randomized arms equal
		DDP+CTX+ ADR	23	7 (30)	3 (13)	–	–	ALL–13.5			
Ehrlich [75]	1982	DDP+VBL+BLEO	7	3 (43)	2 (29)	–	–	–	–	–	Regimen based upon clonogenic assay data, regimen active but too toxic
Lund [76]	1982	DDP+HEX	29	10 (34)	2 (7)	6	4	–	–	–	
Talley [77]	1983	DDP+MTX	26	7 (27)	4 (15)	18+	–	–	–	–	Escalating doses of MTX
Surwit [78]	1983	DDP+VBL+ BLEO+HEX	35	17 (50)	8 (23)	13.5	10	16+	12.3+	5.8	Clonogenic assay guided study, felt to be active

DDP = cisplatin, HEX = hexamethylmelamine, ADR = adriamycin, CTX = cyclophosphamide, 5FU = 5-fluorouracil, VBL = vinblastine, BLEO = bleomycin, MTX = methotrexate; CR = complete response, PR = partial response, NR = no response, DFI = disease free interval.

The toxicity of these combinations is generally tolerable. Approximately one-third of the patients develop significant myelosuppression with a small percentage requiring hospitalization for infectious complications. Toxicity becomes intolerable in patients who have been more aggressively treated with radiation therapy or multiagent chemotherapy regimens [68]. This group of patients do poorly with the cisplatin based salvage regimens and generally have short survivals.

Combination chemotherapy regimens in untreated patients that include cisplatin

Non-randomized studies

Based upon improved responses and survivals in patients resistant to prior alkylator treatment, the next logical step was to use cisplatin combination chemotherapy as initial treatment for ovarian carcinoma. The last 3 years have seen an avalanche of such studies. Most are early and difficult to evaluate because they are based only upon response. Tables 4 and 5 list cisplatin and multiagent data, current to July, 1983. Response rates for these regimens, both in randomized and non-randomized studies range from 29% to 92%. There appears to be an appreciable increase in the clinical complete response rates. Complete response durations have lasted 4 years or greater, but the median response duration from investigations with long enough follow-up are approximately 20 months. Partial response durations of 6 to 12 months do not differ from those obtained from other single or multiagent regimens. Median survivals of patients with complete responses in many studies are not yet reached at 24+ months. Overall survivals range in duration from 14 months to 36+ months depending upon the regimen and pretreatment variables of the patient population treated.

We believe that our nonrandomized pilot experience with an aggressive, multiagent, first line treatment regimen containing cisplatin, cyclophosphamide, adriamycin, and hexamethylmelamine (Hexa-CAP) [100, 101] is representative of the many studies listed in Table 4. We began treating all patients with Stages II, III, and IV ovarian carcinoma soon after aggressive initial debulking surgery with a regimen consisting of cisplatin 60 mg/m^2 intravenously on day 1, adriamycin 20 mg/m^2 intravenously on days 1 and 8, cyclophosphamide 350 mg/m^2 intravenously on days 1 and 8, and hexamethylmelamine 450 mg/m^2 daily by mouth on days 1 to 14. This intensive regimen is given monthly for 6 months, then discontinued. Following discontinuation of treatment, clinical reevaluation and whenever possible, a thorough second look laparotomy was performed.

We now have long term evaluation data (minimum followup of 35

Table 4. Cisplatin containing chemotherapy combination in patients with untreated ovarian carcinoma: Nonrandomized studies

Author	Yr	Drugs	Evaluable pts	Response (%)	CR (%)	pCR (%)	Median DFI (months)		Median survival (months)			Comments
							CR	PR	CR	PR	NR	
Parker [82]	1983	DDP+CTX+ ADR	55	—	30 (55)	11/30 (37)	—	—	36	—	—	Long term survival (36+mos) in pts NED after 2nd look laparotomy
Griffin [83]	1983	DDP+ADR sequential with CTX+ HEX+5FU	44	35 (80)	35 (80)	5/16 (31)	—	—	18+	—	—	Series with minimal follow-up
Rosso [84]	1983	DDP+MTX+ 5FU	38	23 (61)	16 (45)	8/16 (50)	—	—	12 mos – 54% 18 mos – 46%			
Schulman [85]	1983	DDP+CTX+VBL+ HEX	17	5 (29)	2 (13)		5+, 20+	4+, 11+, 16+	—	—	—	Very early data
Goldhirsch [86]	1983	DDP+LPAM+ HEX	25	18 (72)	10 (40)	10/18 (40)	8+	—	—	—	—	All pts with CR surgically confirmed
Stiff [87]	1983	DDP+CTX+ ADR	28	23 (82)	23 (82)	12/23 (43)	21	—	—	—	—	Short course (6 treatment cycles)
Steiner [88]	1983	DDP+ADR	31	23 (74)	18 (58)	5/9 (56)			72% at 24 mos			Patients with CR treated with abdominal radiation
Williams [89]	1982	DDP+CTX+ ADR then CLB	35	28 (80)	21 (68)	2/7 (29)	26.5	6	—	—	—	Pilot trial suggested combination is useful
Fuks [90]	1982	DDP+CTX+ADR+ HEX	13	8 (83)	5 (38)	3/10 (30)	—	—	—	—	—	Pilot trial evaluating radiation therapy after induction chemotherapy
Vogl [91]	1983	DDP+CTX+ ADR+HEX	26	19 (82)	11 (42)		21.5	6.5	2 cm–38+ 2–10 cm 33.9			Improved response and survival for <2 cm residual tumor, no surgical confirmation of CR

DDP = cisplatin, CTX = cyclophosphamide, ADR = adriamycin; HEX = hexamethylmelamine, 5FU = 5-fluorouracil, VBL = vinblastine, MTX = methotrexate; CR = clinical complete response, pCR = pathologic complete response (documented by laparotomy), PR = partial response, NR = no response, DFI = disease free interval, NED = no evidence of disease.

Table 4. (continued)

Author	Yr	Drugs	Evaluable pts	Response (%)	CR (%)	pCR (%)	Median DFI (months)		Median survival (months)			Comments
							CR	PR	CR	PR	NR	
Israel [92]	1983	DDP+CTX	20	15 (75)	7 (47)	–	–	–	25+	8	–	
Wernz [93]	1982	DDP+CTX	19	14 (74)	7 (37)		13+	–	12+	10	–	
Young JA [94]	1982	DDP+CTX+ ADR Alternating with CTX+ MTX+5FU	29	19 (66)	14 (48)	6/20 (30)	–	–		ALL – 19.5		
Budd [95]	1982	DDP+CTX+ ADR	36	30 (83)	18 (50)		–	–		19+, ALL – 21		Variable doses of adriamycin and cisplating
Chung [96]	1982	DDP+CTX+ADR+HEX	20	14 (70)	4 (20)	6/8 (75)	–	12				
Hernandez [97]	1983	DDP+CTX+HEX Alternating with HEX+ CTX+ADR	18	10 (55)	4 (22)			ALL – 9		ALL – 14		Predominant bulky residual disease (> 2 cm)
Razis [98]	1982	DDP+CTX+ ADR+HEX	38	31 (79)	12 (32)		–	–	–	–	–	Pilot data suggesting antitumor efficacy
Young RC [99]	1982	DDP+CTX+ HEX+5FU	51	38 (75)	21 (41)	10/21 (48)	–	24+	17.5	6	–	DDP increases CR rates in pts with minimal residual disease
Greco [100]	1981	DDP+CTX+ DOX+HEX	46	44 (96)	35 (80)	14/37 (38)		ALL 17+		ALL 19+		Short course, intensive treatment, high response rate due to good prognosis patients

DDP = cisplatin, CTX = cyclophosphamide, ADR = adriamycin; HEX = hexamethylmelamine, 5FU = 5-fluorouracil, VBL = vinblastine, MTX = methotrexate; CR = clinical complete response, pCR = pathologic complete response (documented by laparotomy), PR = partial response, NR = no response, DFI = disease free interval, NED = no evidence of disease.

Table 5. Cisplatin containing chemotherapy combination in patients with untreated ovarian carcinoma randomized studies

Author	Yr	Drugs	Evaluable pts	Response (%)	CR (%)	pCR	Median DFI (months) CR	Median DFI (months) PR	Median survival (months) CR	Median survival (months) PR	Comments
Williams [102]	1983	DDP+CTX+ ADR	27	18 (72)			–	–	ALL – 17		Pts failing one arm, crossed to the other, significant increase in response rate but no survival advantage
		CLB	27	6 (26)			–	–	ALL – 17		
Decker [103]	1982	CTX	19	5 (26)	3 (16)	1/3 (33)	ALL – 7.5		ALL – 16.8		Two drug arm superior
		DDP+CTX	21	16 (76)	12 (57)	5/10 (50)	ALL – 28		ALL – 36+		
Carmo-Pereira [104]	1983	CTX	27	18 (66)	12 (44)	6/9 (67)	ALL – 11		ALL – 11		No difference in 2 arms
		DDP+ADR+HEX	26	10 (38)	5 (19)	3/5 (60)	ALL 10		ALL – 12		
Sturgeon [105]	1982	LPAM	38		5 (13)		–	–	–	–	No difference in survival all arms, progression free survival advantage for DDP+CTX+ADR
		CTX+MTX+ 5FU+HEX	37		7 (19)		–	–	–	–	
		DDP+CTX+ ADR	40		12 (30)		–	–	–	–	
Vogl [106]	1982	LPAM	118	53 (45)	24 (20)		ALL – 8.5		ALL – 18		Four drugs superior for response >2 cm residual disease, age >50 yrs; no difference in survival
		DDP+CTX+ ADR+HEX	127	81 (67)	52 (41)		ALL – 13.9		ALL – 18		
Bell [107]	1982	CLB	19	3 (23)	ALL – 7		ALL – 33+		8		Improved response to combination, no survival advantage
		CTX+DDP	17	9 (69)	ALL – 13		ALL – 26+		8		
Bruckner [108]	1981	DDP	18	4 (22)	1 (6)		ALL – 9		ALL – 19		Improved response and survival with DDP regimens
		DDP+ADR	18	12 (68)	6 (33)		ALL – 15		ALL – 20		
		TTP+MTX	17	5 (29)	1 (6)		ALL – 3		ALL – 11		
Neijt [109]	1983	DDP+CTX+ ADR+HEX	99	79 (80)	32 (40)	24 (30)	–	–	–	–	DDP combination superior to non-DDP containing combination for response and CR rate
		CTX+MTX+ HEX+5FU	97	50 (52)	18 (19)	9 (17)	–	–	–	–	

DDP = cisplatin, CTX = cyclophosphamide, ADR = adriamycin, CLB = chlorambucil, HEX = hexamethylmelamine, MTX = methotrexate, LPAM = L-phenylalmine mustard (melphalan), 5FU = 5-fluorouracil, TTP = ThioTEPA; CR = clinical complete response, pCR = pathologic complete response (documented by laparotomy), PR = partial response, NR = no response, DFI = disease free interval.

Table 5. (continued)

Author	Yr	Drugs	Evaluable pts	Response (%)	CR (%)	pCR	Median DFI (months) CR	Median DFI (months) PR	Median survival (months) CR	Median survival (months) PR	Comments
Imholz [110]	1983	DDP+CTX+ ADR	21	12 (57)	12 (57)		75% 18 mo		75% at 18 mos		
		CTX+5FU+ ADR	37	24 (65)	24 (65)		57% at 18 mos		60% at 18 mos		
Omura [111]	1982	CTX+ADR	100	46 (46)	20 (20)		ALL - 9.5		–	–	Three drugs improve complete response rate
		DDP+CTX+ADR	91	45 (70)	40 (44)		ALL - 15.0		–	–	
Edwards [112]	1983	CTX+ADR+ HEX	84	22 (26)	20 (24)	14/20 (70)	–	–	ALL - 26.4		No difference in arms based on survival
							–	–			
		DDP+LPAM	74	31 (42)	27 (34)	20/27 (74)	–	–	ALL - 29.6		
Pouillart [113]	1982	CTX+ADR+5FU	47	29 (62)	15 (32)		–	–	ALL - 24		No difference in arms
		DDP+CTX+ ADR+5FU	24	16 (66)	8 (33)		–	–	ALL - 21		
Stehman [114]	1983	DDP+CTX+ ADR (DDP Day 1) DDP+CTX+ ADR (DDP Days 1-5)	56	44 (79)	23 (41)		33	12.7	33	20	No difference between arms, data analyzed as one group
Bruckner [115]	1983	DDP+ADR	NA	(60)	(19)		ALL - 21		ALL - 25		No difference between treatment arms
		DDP+CTX+ ADR	NA	(52)	(18)		ALL - 24		ALL - 27		
Barker [116]	1981	DDP+CLB	46	24 (52)	13 (28)		31	8	28	10	Arms are equal
		DDP+CLB+ADR	39	21 (54)	11 (28)		24	9	26+	8	
Bruckner [117]	1983	DDP+ADR	20	9 (43)	7 (36)		–	–	ALL - 18		Four drugs superior to 2 drugs in patients with poorly differentiated tumors
		DDP+CTX+ ADR+HEX	37	21 (57)	10 (26)		–	–	ALL - 25		

DDP = cisplatin, CTX = cyclophosphamide, ADR = adriamycin, CLB = chlorambucil, HEX = hexamethylmelamine, MTX = methotrexate, LPAM = L-phenylalmine mustard (melphalan), 5FU = 5-fluorouracil, TTP = ThioTEPA; CR = clinical complete response, pCR = pathologic complete response (documented by laparotomy), PR = partial response, NR = no response, DFI = disease free interval.

months, range 35–58 months) for the first 58 patients treated with this combination. Twenty-one (36%) of the 58 had limited residual disease while 37 had bulky disease (as defined by residual tumor >3 cm). All 21 patients with limited residual disease had second look laparotomies. Seventeen (81%) of these 21 patients had a complete pathologic response. Five (29%) subsequently relapsed (17 to 28 months) and 12 remain in remission from 35 to 58 months since the beginning of chemotherapy.

Of 37 patients with bulky residual disease, 26 (78%) had a second look laparotomy for treatment evaluation. Two patients (5%) had pathologic complete responses and three (8%) had clinical complete responses. Two patients (5%) did not respond to chemotherapy and the rest (81%) had partial responses. Twenty-six (78%) of the 37 patients are dead from their ovarian carcinoma. Eight (22%) of 37 are alive with known disease. One patient with a pathologic complete response died of breast cancer without evidence of ovarian carcinoma. Two patients, one with a pathologic complete response and one with a clinical complete response are alive at 44 and 60 months following treatment.

We have previously reported a high overall (96%) response rate with our regimen [100]. Our data suggest that the pretreatment tumor burden has major effects upon long term survival which is primarily associated with a pathologic complete response. Patients with partial response, while clearly benefitting from the treatment, do not obtain long term (>24 months) responses or survivals. Therefore, only patients with *complete* responses have improved survivals and, in a small percentage (12/58–21%), have long term survivals of 4 years or greater.

The efficacy of multiagent, cisplatin based regimens, impressive as they appear, need further evaluation. Most of these regimens induce more toxicity, primarily due to the marked increase in nausea and vomiting, myelotoxicity, and nephrotoxicity attributible to cisplatin [112]. While most patients are able to tolerate the additional toxicity, the newer regimens need justification based upon improved remission durations and survival in randomized trials with less toxic regimens.

Randomized studies – cisplatin combination vs single agent alkylator

Table 5 lists a large group of recently reported, mostly in abstract form, ongoing, randomized trials which are attempting to compare cisplatin and non-cisplatin containing treatment. Data published by groups led by Decker [103], Williams [102], Carmo-Pereira [109], Sturgeon [105], Vogl [106], and Bell [107] (Table 4) all address the problem of comparing single agent alkylator therapy to cisplatin based combination chemotherapy. Response

data in these series generally show superiority for the multiagent, cisplatin containing treatment arm. Survival, however, with the exception of the study of Decker *et al.* [103] is no different between the single alkylating agent and cisplatin combination. Decker's study [103] is a well balanced group of 40 patients, 23 (58%) patients had residual tumor > 2 cm prior to treatment. Response data were reported as pathologic complete response, progression free interval, and survival. In all parameters, there is an advantage for the multiagent arm. More recently, a large scale cooperative study (253 patients entered, 246 evaluable) from the Eastern Cooperative Oncology Group (ECOG) suggests no difference in survival or disease free interval despite a higher response rate in the cisplatin combination chemotherapy arm [106]. Similar results to these are reported by Sturgeon [105], Williams [102], Carmo-Pereira [104] and Bell [107]. With longer follow-up, survival differences may be appreciated.

The reason for this discrepancy probably lies in the multiple pretreatment prognostic factors which are commonly associated with response and survival in ovarian carcinoma patients. Of primary importance is residual tumor bulk. Most studies suggest that patients with tumor mass of 2 cm or less have prolonged survival and higher response rates to chemotherapy. Decker's study [103], had a higher percentage of patients with minimal residual tumor (less than 2 cm) (42%) compared to other studies [(13%) in Bell *et al.*, (20%) in Vogl *et al.*, and (19%) in Carmo-Pereira *et al.*]. It is becoming clear that complete responses are the only responses that provide long term survival (> 36 months) [101, 112]. In addition, there is evidence to suggest that multiagent chemotherapy regimens, particularly those containing cisplatin or those with aggressive, high dose treatment, are able to induce higher percentages of complete pathologic responses in patients with minimal residual tumor prior to treatment [100, 101, 103, 112]. Therefore, in order to observe a statistically higher survival rate, a larger sample of patients with minimal residual disease must be treated if the 20–30% of these patients who will obtain complete pathologic responses and become long term survivors are to affect the overall survival curves. The only other way to evaluate this phenomenon is to have a large group of patients (e.g. the ECOG study of Vogl *et al.*; stratify and analyze the data of patients with tumor residual at initial treatment of 2 cm or less. Preliminary analysis at 3 years of 70 such patients suggests *no* statistical difference in complete response rate or survival between LPAM and cisplatin, cyclophosphamide, adriamycin, and hexamethylmelamine treated patients [119]. These data are provocative because they suggest no difference in survival with a cisplatin based multiagent regimen when compared to melphalan, even in patients with minimal residual tumor postoperatively. We await further analysis regarding total dose administered, surgical information, and prognostic factors, in the long-term surviving patients.

We have noted that in many studies, patients receiving multiagent chemotherapy, particularly those containing cisplatin have higher initial response rates, high complete clinical responses, yet no difference in survival when compared to patients receiving single agents – an apparent paradox. Since most of the patients treated in these studies have greater than 2 cm tumor bulk, most complete clinical responses are probably not pathologic complete responses. The median durations of such responses are 12 to 30 months. The duration of clinical responses to single agent alkylator therapy may be short; but, as has been previously reviewed, many patients relapsing from single agent alkylator therapy will respond to second-line treatment with cisplatin containing regimens. In many of these studies [102, 105, 107, 109, 112], relapsed patients were crossed to the opposite treatment arm. Therefore, most relapsed, alkylator-treated only patients received cisplatin combinations. Those patients who relapsed at a later time following combination chemotherapy, do not respond to further therapy. Hence, survivals for both patient groups become equivalent.

Variables other than residual tumor

Ozols *et al.* observed that histologic tumor grade plays an important role in determining those patients most likely to benefit from multiagent chemotherapy [120]. Most single arm and randomized studies with cisplatin have not detected such an advantage. Recently, Bruckner *et al.* [117] and Vogl *et al.* [119] have observed that patients with poorly differentiated tumors have improved response rates and survivals than other patients. Decker *et al.* [103] have observed a better survival in patients with well differentiated tumors, but also found that younger patients more frequently had well differentiated tumors.

Most investigators agree that patients aged less than 50 years have a better prognosis than those older than 50 years. Reasons for better survivals in young patients may include a better performance status at initial treatment, lower grade tumor, the ability to tolerate higher doses of chemotherapy for a longer period of time and the ability to tolerate more aggressive surgery because of fewer preoperative medical problems. Tumor histology appears to minimally influence the induction of surgical complete remissions.

Little information relating the percent of optimal dose administered to response and survival exists. This may be a very important variable if the previously discussed dose-response relationships exist. We have found that most patients responded to treatment when they received a higher percent of the total optimal dose, particularly in the first 3 cycles of treatment [121].

Randomized studies – cisplatin vs non-cisplatin multiagent combinations

Bruckner's randomized study of cisplatin versus cisplatin and adriamycin versus thioTepa-methotrexate suggests superiority for platinum regimens. Little difference in response and survival between cisplatin alone and cisplatin and adriamycin was found. The small numbers of patients in each of the arms make statistical conclusions difficult. It is surprising that only 29% of patients treated with ThioTepa-methotrexate responded with a median survival of 11 months. Previous data from the same institution reported a response rate of 67% with median survival of 17.4+ months (Table 1, Greenspan 1979) [32]. Improved diagnostic radiology procedures may account for such large differences.

A number of investigators (Table 5, Neijt, 1983, Imholz, 1982, Omura, 1982, Sturgeon, 1982, Edwards, 1982, Pouillant, 1982) [105, 110–113] have reported preliminary data comparing cisplatin combinations to non-cisplatin containing multiagent combinations. In most of these studies we find the same situation encountered in the evaluation of cisplatin and single agent treatment; that is, high initial response rates for the cisplatin combination including higher complete response rates. In most studies, the follow-up evaluation period is too short to determine whether cisplatin combinations will improve survivals.

Of interest is the recently published study of Edwards *et al.* [112]. Particular emphasis was paid to surgical technique in this large, well randomized and balanced investigation. Initial operation in both arms rendered 44% of patients with residual tumor of less than 2 cm. All patients with complete clinical response underwent second-look laparotomy. The results of these procedures suggest *equal* therapeutic efficacy for the cisplatin and noncisplatin multiagent regimens compared. No statistical advantages were found for response rates, complete response rates, or survival for either arm. The relatively low response rates, yet long survival durations are probably due to the group of patients in both arms who achieved pathologic complete responses and are long term survivors. Toxicity, in the form of myelotoxicity was significantly greater for the platinum combination. The dose reductions on the cisplatin arm (68% of cisplatin combination patients receiving reduced doses by the fourth treatment cycle versus 24% of non-cisplatin patients) may explain the relatively low response rate to the cisplatin combination as compared to other published regimens. Whether such dose reductions are important determinants of complete pathologic response and survival needs further investigation. Preliminary data from the 2 armed GOG study (Omura, 1982) [111] suggest response rate, response duration, and progression free interval advantage to the 3 drug cisplatin regimen when compared to the 2 arm adriamycin-cyclophosphamide combination. Preliminary survival data [133] favor the 3 drug cisplatin arm in patients with

measurable disease. No survival advantage has been shown in patients with bulky but non-measurable disease.

Chemoimmunotherapy

The Southwest Oncology Group compared cyclophosphamide plus doxorubicin with and without Bacillus Calmette-Guerin immunotherapy [122]. Preliminary results indicated statistically significant improvement in overall response rates and survival for the chemoimmunotherapy group which could not be explained by imbalances in the various stratifications or prognostic factors. In a non-randomized study, the GOG [123] evaluated C-Parvum in combination with melphalan. Data from this study suggested some advantage to C-Parvum, however, a subsequent randomized multiarm GOG study did not suggest any advantage to the addition of C-Parvum [51]. It does not appear that nonspecific immunotherapy has much to offer patients with advanced ovarian carcinoma.

Summary

In spite of a number of chemotherapeutic agents with significant antitumor activity in epithelial carcinoma of the ovary, most patients who are diagnosed with advanced stage (FIGO stage III and IV) will eventually succumb to their tumor.

A critical review of multiagent chemotherapy reveals the importance of initial staging and debulking surgery. Most patients who achieve a complete pathologic response start chemotherapy with minimal residual tumor, that is, no tumor nodules remaining 2 cm or greater in size. Response is not a clear endpoint. Criteria of response are not uniform and a wide variety of definitions exist. Because advanced ovarian carcinoma does not lend itself to clinical evaluation, comparisons of studies based upon 'complete clinical response' are misleading. Surgery, therefore, has evolved as a major means of determining response. In many series, 50% to 70% of patients with complete pathologic response enjoy long term remissions.

The question that oncologists must answer is how to achieve a complete pathologic response in patients with advanced ovarian carcinoma. Non-platinum containing combination chemotherapy, when given aggressively, may improve survival based upon complete pathologic responses in patients with limited residual disease after primary resection. For patients with larger tumor burdens, non-platinum containing combination chemotherapy does not provide improved survival over single agents. The addition of cisplatin to standard combinations increases toxicity and may improve the percentage of complete pathologic responders in patients with minimal resi-

dual tumor. In patients with large bulk tumor, the addition of cisplatin improves the initial response rate to treatment, but does not improve survival over standard single agent alkylating agent treatment or standard non-platinum containing regimens. This lack of survival improvement is most likely due to the ability of cisplatin based multiagent regimens to induce responses for 6 to 12 month durations in patients who have relapsed after prior single alkylating agent therapy. Large randomized studies underway will compare cisplatin based multiagent regimens to non-cisplatin containig, less toxic, multiagent combinations (such as adriamycin-cyclophosphamide and Hexa-CAF). The relationship of dose, degree of dose reductions, and timing of dose reductions to complete pathologic response and survival need to be analyzed and studied in more detail.

Future directions

New chemotherapy agents are constantly being tested in patients with advanced ovarian carcinoma. Since most ovarian carcinomas are resistant to secondary therapy of any type after initial multiagent chemotherapy, standard Phase II trials of new agents may not identify active new drugs. The human tumor colony stem cell assay will help improve this situation by testing sensitive human ovarian cells *in vitro* with new agents, while allowing standard multiagent therapy to be administered to patients without exposing them, at initial therapy, to Phase II drugs. Among the new compounds in preclinical and clinical evaluation are new platinum analogues which are less toxic, and *in vitro*, are equally effective [124, 125].

Intraperitoneal drug administration has been explored with single agents [126–131]. Recent data [132] has suggested that multiagent intraperitoneal instillation can be tolerated by patients. The common presence of postoperative abdominal adhesions with loculation of abdominal spaces makes equal distribution of intraperitoneal drugs difficult. In spite of these problems, further data will help define the use of intraperitoneal drug administration.

Finally, biologic response modifiers promise new approaches to cancer therapy in the future. Interferons and specific monoclonal antibodies as examples with or without combination cytotoxic therapy may promise increases in pathologic complete responses and survivals.

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References

1. Silverberg E, Lubera JA: Cancer statistics, 1983. CA 33:2–25, 1983.
2. DeVita VT, Wasserman TH, Young RC, Carter SK: Prospectives on research in gynecologic oncology. Cancer 38:509–525, 1976.
3. Skipper HC, Chapman JB, Bell M: The anti-leukemic action of combinations of certain known anti-leukemia agents. Cancer Res 11:109–114, 1952.
4. Johnson RK, Goldin A: The clinical impact of screening and other experimental tumor studies. Cancer Treat Rev 2:1, 1975.
5. Bruns BC, Rutledge FN, Gallager HS: Phenylalanine mustard in the palliative management of carcinoma of the ovary. Obstet Gynecol 22:30–37, 1963.
6. Decker DG, Mussey E, Malkasian GD, Johnson CE: Cyclophosphamide in the treatment of ovarian cancer. Clin Obstet Gynecol 11:382–400, 1968.
7. Wallach RC, Kabakow B, Blinick G, Antopol W: Thio-Tepa chemotherapy for ovarian carcinoma: Influence of remission and toxicity on survival. Obstet Gynecol 35:278–280, 1970.
8. Rossof AH, Drukker BH, Talley RW, Torres J, Bonnett J, Brownlee RW: Randomized evaluation of chlorambucil and melphalan in advanced ovarian cancer. Proc Am Soc Clin Oncol 17:300, 1976.
9. Bruckner HW, Cohen CJ, Wallach RC, *et al.*: Treatment of advanced ovarian cancer with cis-dichlorodiammineplatinum (II): poor risk patients with intensive prior therapy. Cancer Treat Rep 62:555–558, 1978.
10. Wiltshaw E, Subramarian S, Alexopoulo C Barker GH: Cancer of the ovary: a summary of experience with cis-dichlorodiaminomineplatinum (II) at the Royal Marsden Hospital. Cancer Treat Rep 63:1545–1548, 1979.
11. Young RC, Von Hoff DD, Gormley P, *et al.*: Cis-dichlorodiammineplatinum (II) for the treatment of advanced ovarian cancer. Cancer Treat Rep 63:1539–1544, 1979.
12. Johnson BL, Fisher RI, Bender RA, DeVita VT, Chabner BA, Young RC: Hexamethylmelanine in alkylating agent-resistant ovarian carcinoma. Cancer 42:2157–2161, 1978.
13. Bonomi PD, Mladineo J, Morrin B, Wilbanks G, Slayton RE. Phase II trial of hexamethylmelamine in ovarian carcinoma resistant to alkylating agents. Cancer Treat Rep. 63:137–138, 1979.
14. Bonadonna G, Beretta G, Tancini G, DePalo GM, Gasparini-Roberto Doci M: Adriamycin as a single agent in various forms of advanced neoplasia of adults and children. Tumor 60:373–391, 1974.
15. O'Bryan RM, Luce JK, Talley RW, Gottleib JA, Baker LH, Bonadonna G: Phase II evaluation of adriamycin in human neoplasia. Cancer 32:1–8, 1973.
16. Bolis G, D'Incalci M, Gramellini F, Mangioni C: Adriamycin in ovarian cancer patients resistant to cyclophosphamide. Eur J Cancer 14:1401–1402, 1973.
17. Bolis G, D'Incalci M, Belloni C, Mangioni C: Hexamethylmelamine in ovarian cancer resistant to cyclophosphamide and adriamycin. Cancer Treat Rep 63:1375–1377, 1979.
18. Schwartz PE: Surgical management of ovarian cancer. Arch Surg 116:99–106, 1981.
19. Young RC, Wharton JT, Decker DG, Piver MS, Edwards B, McGuire WP. Staging laparotomy in early ovarian cancer. Proc Am Soc Clin Oncol 20:399, 1979 (Abstract).

20. Rosenoff SH, DeVita VT, Hubbard S, Young RC. Peritoneoscopy in the staging and follow-up of ovarian cancer. Semin Oncol 2:223–228, 1975.
21. Griffiths CT, Parker LM, Fuller AF: Role of cytoreductive surgical treatment in the management of advanced ovarian cancer. Cancer Treat Rep 63:235–240, 1975.
22. Julian CG, Woodruff JD: The biologic behavior of low-grade papillary serous carcinoma of the ovary. Obstet Gynecol 40:860–867, 1972.
23. Decker DG, Malkasian GD, Taylor WF. Prognostic importance of histology grading in ovarian carcinoma. Natl Cancer Inst Monogr 42:9–11, 1975.
24. Ozols RF, Garvin AJ, Costa J, Simon RM, Young RC. Advanced ovarian cancer. Correlation of histologic grade with response to therapy and survival. Cancer 45:572–581, 1980.
25. Hubbard SM, Barkes P, Young RC: Adriamycin therapy for advanced ovarian carcinoma recurrent after chemotherapy. Cancer Treat Rep 62:1375–1377, 1978.
26. Stanhope CR, Smith JP, Rutledge F: Second trial drugs in ovarian cancer. Gynecol Oncol 5:52–58, 1977.
27. Whitley N, Brenner D, Francis A, *et al.*: Use of the computed tomographic whole body scanner to stage and follow patients with advanced ovarian carcinoma. Invest Radiol 16:479–486, 1981.
28. Brenner DE, Grosh WW, Jones HW, *et al.*: An evaluation of the accuracy of computed tomography in patients with ovarian carcinoma prior to second look laparotomy. Proc Am Soc Clin Oncol 2:149, 1983 (abstract).
29. Smith JP, Delgado G, Rutledge FN: Second look operation in ovarian carcinoma. Cancer 38:1438–1442, 1976.
30. Schwartz PE, Smith JP: Second look surgery in ovarian cancer management. Am J Obstet Gynecol 138:1124–1130, 1980.
31. Ehrlich CE, Einhorn LH, Stehman FB, Roth L: Response, 'second look' status and survival in stage III-IV epithelial ovarian cancer treated with cis-dichlorodiammineplatinum: (II), adriamycin, and cyclophosphamide. Proc Am Soc Clin Oncol 21:423, 1980 (abstract).
32. Greenspan EM: ThioTepa and methotrexate chemotherapy of advanced ovarian carcinoma. J Mt Sinai Hosp NY 35:52–67, 1968.
33. Smith JP, Rutledge F: Chemotherapy in the treatment of cancer of the ovary. Amer J Obstet Gynecol 107:691–700, 1970.
34. Lokich JJ, Skarin AT: Five-drug combination chemotherapy for dissiminated adenocarcinoma. Cancer Chemother Rep 56:761–787, 1972.
35. Parker LM, Griffiths T, Yankel RA, *et al.*: Combination chemotherapy with adriamycin-cyclophosphamide for advanced ovarian carcinoma. Cancer 46:669–674, 1980.
36. Neijt JP, Van Lindert ACM, Vendrik CAJ, *et al.*: Treatment of advanced ovarian carcinoma with a combination of hexamethylmelamine, cyclophosphamide, methotrexate, and 5-fluorouracil (Hexa-CAF) in patients with and without previous treatment. Cancer Treat Rep 64:323–326, 1980.
37. DePalo G, Demicheli R, Valagussa P, *et al.*: Prospective study with HEXA-CAF combination in ovarian carcinoma. Cancer Chemother Pharmacol 5:157–161, 1981.
38. Fiorentino MY, Fosser V, Tredese F, *et al.*: Combined chemo-surgical treatment for stage III ovarian cancer. Chemioterapia 1:159–163, 1982.
39. Jobson VW, Nordqvist SRB, Averette HE: Advanced and recurrent carcinoma of the ovary treated with a combination of cyclophosphamide, doxorubicin, and cytosine arabinoside. Gynecol Oncol 15:154–159, 1983.
40. Greenspan EM, Fieber M: Combination chemotherapy of advanced ovarian carcinoma with the antimetabolite methotrexate and the alkylating agent, Thio-TEPA. J Mt Sinai Hosp NY 29:48–58, 1962.
41. Smith JP, Rutledge FN: Random study of hexamethylmelamine, 5-fluorouracil, and mel-

phalan in treatment of advanced carcinoma of the ovary. Natl Cancer Inst Monogr 42:169–172, 1975.
42. Barlow JJ, Piver MS: Methotrexate (NSC 740) with citrovorum factor (NSC 3590) rescue, alone and in combination with cyclophosphamide (NSC 26271) in ovarian cancer. Cancer Treat Rep 60:527–533, 1976.
43. Barlow JJ, Piver MS: Single agent vs combination chemotherapy in the treatment of ovarian cancer. Obstet Gynecol 49:609–611, 1977.
44. DePalo GM, DeLena M, Bonadonna G: Adriamycin versus adriamycin plus mephalan in advanced ovarian carcinoma. Cancer Treat Rep 61:355–357, 1977.
45. Brodovsky HS, Temrin N, Sears M: Melphalan versus cyclophosphamide, methotrexate and 5-fluorouracil in women with ovarian cancer. Proc Am Soc Clin Oncol, 18:308, 1977 (abstract).
46. Young RC, Chabner BA, Hubbard SP, *et al.*: Advanced ovarian adenocarcinoma. A prospective clinical trial of melphalan (L-PAM) versus combination chemotherapy. N Engl J Med 299:1261–1266, 1978.
47. Bruckner HW, Pagano M, Falkson G, *et al.*: Controlled prospective trial of combination chemotherapy with cyclophosphamide, adriamycin, and 5-fluorouracil for the treatment of advanced ovarian cancer: a preliminary report. Cancer Treat Rep 63:297–299, 1979.
48. Klaasen DJ, Boyes DA, Gerulath A, *et al.*: Preliminary report of a clinical trial of the treatment of patients with advanced stage III and IV ovarian cancer with melphalan, 5-fluorouracil, and methotrexate in combination and sequentially: a study of the clinical trials group of the National Cancer Institute of Canada. Cancer Treat Rep 63:289–295, 1979.
49. Edmonson JH, Fleming TR, Decker DG, *et al.*: Different chemotherapeutic sensitivities and host factors affecting prognosis in advanced ovarian carcinoma versus minimal residual disease. Cancer Treat Rep 63:241–247, 1979.
50. Park RC, Blom J, DiSaia PJ, Lagasse LD, Blessing JA. Treatment of women with disseminated or recurrent advanced ovarian cancer with melphalan alone, in combination with 5-fluorouracil and dactinomycin, or with the combination of cytoxan, 5-fluorouracil and dactinomycin. Cancer 45:2529–2542, 1980.
51. Barlow JJ, Piver MS, Lele SB: High-dose methotrexate with 'rescue' plus cyclophosphamide as initial chemotherapy in ovarian adenocarcinoma. Cancer 43:1333–1338, 1980.
52. Turbow MM, Jones H, Yu VK, Greenberg B, Hannigan J, Torti FM: Chemotherapy of ovaraian carcinma: a comparison of melphalan vs. adriamycin-cyclophosphamide. Proc Am Assoc Cancer Res, 21:196, 1980 (Abstract).
53. Schwartz PE, Lawrence R, Katz ME: Combination chemotherapy for advanced ovarian cancer: a prospective randomized trial comparing hexamethylmelamine-cyclophosphamide to adriamycin-cyclophosphamide. Cancer Treat Rep 65:137–141, 1981.
54. Medical Research Council's working party on ovarian cancer: Medical Research Council study on chemotherapy in advanced ovarian cancer. Br J Obstet Gynecol 88:1174–1185, 1981.
55. Trope C for Swedish Cooperative Ovarian Cancer Study Group: A prospective and randomized trial of melphalan vs adriamycin-melphalan in advanced ovarian carcinoma. Proc Am Soc Clin Oncol 22:469, 1981 (abstract).
56. Omura GA, Morrow CP, Blessing JA *et al.*: A randomized comparison of melphalan versus melphalan plus hexamethylmelamine versus adriamycin plus cyclophosphamide in ovarian carcinoma. Cancer 51:783–789, 1983.
57. Greenspan EM, Bruckner HW: Comparison of remission induction with triethylene thiophosphoramide or methotrexate in bulky stage IIIb ovarian cancer. Natl Cancer Inst Monogr 42:173–175, 1975.
58. Hande KR, Oldham RK, Fer MF, *et al.*: Randomized study of high-dose versus low-dose methotrexate in the treatment of intensive small cell lung cancer. Amer J Med 73:413–419,

1982.
59. Corbett TH, Griswold DP, Mayo JG, *et al.*: Cyclophosphamide-adriamycin combination chemotherapy of transplantable murine tumors. Cancer Res 35:1568–1573, 1975.
60. Henderson IC, Canellos GP: Cancer of the breast: the past decade. N Engl J Med 302:17–30, 78–90, 1980.
61. Greco FA, Oldham RK: Small cell lung cancer. N Engl J Med 301:355–358, 1979.
62. Young RC, Cannellos GP, Chabner BA,: Chemotherapy of advanced ovarian carcinoma: a prospective randomized comparison of phenylalamine mustard and high dose cyclophosphamide. Gynecol Oncol 2:489–497, 1974.
63. Vogl SE, Greenwald E, Kaplan BH, *et al.*: Ovarian cancer: effective treatment after alkylating-agent failure. JAMA 241:1908–1911, 1979.
64. Alberts DS, Hilgers RD, Moon TE, *et al.*: Combination chemotherapy for alkylator-resistant ovarian carcinoma: a preliminary report of a Southwest Oncology Group trial. Cancer Trial Rep 63:301–305, 1979.
65. Kane R, Harvey H, Andrews T, *et al.*: Phase II trial of cyclophosphamide, hexamethylmelamine, adriamycin, and cis-dichlorodiammineplatinum (II) combination chemotherapy in advanced ovarian carcinoma. Cancer Treat Rep 63:307–309, 1979.
66. Wallach RC, Cohen C, Bruckner H, *et al.*: Chemotherapy of recurrent ovarian carcinoma with cis-dichlorodiammine platinum II and adriamycin. Obstet Gynecol 55:371–372, 1980.
67. Vogl SE, Pagano M, Kaplan BH, *et al.*: Combination chemotherapy of advanced ovarian cancer with hexamethylmelamine, cis-platinum, and doxorubicin after failure of prior therapy. Obstet Gynecol 56:635–640, 1980.
68. Piver MS, Lele S, Barlow JJ: Cyclophosphamide, hexamethylmelamine, doxorubicin and cisplatin (CHAD) as second-line chemotherapy for ovarian adenocarcinoma. Cancer Treat Rep 65:149–151, 1981.
69. Bruckner HW, Cohen CJ, Deppe G, *et al.*: Treatment of chemotherapy-resistant advanced ovarian cancer with a combination of cyclophosphamide, hexamethylmelamine, adriamycin, and cis-diamminedichloroplatinum (CHAP). Gynecol Oncol 12:150–153, 1981.
70. Vogl SE, Pagano M, Davis TE, *et al.*: Hexamethylmelamine and cisplatin in advanced ovarian cancer after failure of alkylating agent therapy. Cancer Treat Rep 66:1285–1290, 1982.
71. Sessa C, D'Incalci M, Valente I, *et al.*: Hexamethylmelamine CAF (cyclophosphamide, methotrexate, and 5-FU) and cisplatin-CAF in refractory ovarian cancer. Cancer Treat Rep 66:1233–1234, 1982.
72. Bruckner HW, Cohen CJ, Deppe G, *et al.*: Ovarian cancer: schedule modification and dosage intensification of cyclophosphamide, hexamethylmelamine, adriamycin, and cisplatin regimen (CHAP II). Proc Am Soc Clin Oncol 1:107, 1982 (abstract).
73. Neijt JP, tenBokkel Huinink WW, Hamervma E, *et al.*: Combination chemotherapy including cis-platinum in previously treated patients with advanced ovarian carcinoma. Proc Am Soc Clin Oncol 1:108, 1982 (abstract).
74. Bernath A, Andrews T, Dixon R, *et al.*: Long term followup of HAP (hexamethylmelamine, adriamycin, cis-platinum) vs CAP (cyclophosphamide, adriamycin, cis-platinum) in alkylating agent resistant advanced ovarian carcinoma. Proc Am Soc Clin Oncol 1:110, 1982 (abstract).
75. Ehrlich CE, Stehman FB, Williams SD: Evaluation of vinblastine, bleomycin, and cisplatinum as second trial therapy in patients with recurrent ovarian carcinoma. Proc Am Soc Clin Oncol 1:111, 1983 (abstract).
76. Lund B, Aabo K, Rorth M, Hansen HH: Cis-dichlorodiammine-platinum (II) and hexamethylmelamine in advanced ovarian carcinoma: A phase II study. Eur J Cancer Clin Oncol 18:1089–1092, 1982.
77. Tally R, Boutseleis J, Neidhart JA: Cis-platinum plus high-dose methotrexate: toxicity and

efficacy in ovarian carcinoma. Am J Clin Oncol 6:369–374, 1983.
78. Surwit EA, Alberts DS, Crisp W: Multiagent chemotherapy in relapsing ovarian cancer. Am J Obstet Gynecol 146:613–616, 1983.
79. Hamburger AW, Salmon SE: Primary bioassay of human tumor stem cells. Science 197:461–463, 1977.
80. Hamburger AW, Samon SE, Kim MB, *et al.*: Direct cloning of human ovarian carcinoma cells in agar. Cancer Res 38:3438–3444, 1978.
81. Alberts DS, Chen HS, Salmon SE, *et al.*: Chemotherapy of ovarian cancer directed by the human tumor stem cell assay, Cancer Chemother Pharmacol 6:279–283, 1978.
82. Parker LM, Griffiths CT, Janis D, *et al.*: Advanced ovarian: carcinoma integration of surgical treatment and chemotherapy with cyclophosphamide, adriamycin and cis-diamminedichloroplatin. Proc Am Soc Clin Oncol 2:153, 1983 (abstract).
83. Griffin T, Adarbaum A, Hunter R *et al.*: Alternating combination chemotherapy for advanced ovarian cancer. Proc Am Soc Clin Oncol 2:154, 1983 (abstract).
84. Rosso R, Sertoli MR, Conte PF: Cis-platinum, methotrexate and fluorouracil (PMF) combination chemotherapy in advanced ovarian cancer. Proc Am Soc Clin Oncol 2:155, 1983 (abstract).
85. Schulman P, Budman DR, Wiselberg L, *et al.*: Phase II trial of hexamethylmelamine, cyclophosphamide, vinblastine and cis-platinum (HEXA-CVP) in advanced epithelial ovarian carcinoma. Proc Am Soc Clin Oncol 2:155, 1983 (abstract).
86. Goldhirsch A, Davis B, Greiner R, *et al.*: Different responses of different histological types of ovarian cancer to a combination of cis-platinum, melphalan and hexamethylmelamine. Proc Am Soc Clin Oncol 2:155, 1983 (abstract).
87. Stiff PJ, Lanzotti VJ, Roddick JW: Prolonged combination chemotherapy for ovarian carcinoma does not increase the rate of surgical complete remissions. Proc Am Soc Clin Oncol 2:156, 1983 (abstract).
88. Steiner M, Rubinov R, Borovik R: Multimodel approach (surgery, chemotherapy, radiotherapy) in the treatment of ovarian carcinoma. Proc Am Soc Clin Oncol 2:157, 1983 (abstract).
89. Williams CJ, Mead B, Arnold A *et al.*: Chemotherapy of advanced ovarian carcinoma: initial experience using a platinum based combination. Cancer 49:1778, 1982.
90. Fuks Z, Rizel S, Anteby SO, Biran S: The multimodel approach to the treatment of stage III ovarian carcinoma. Int J Radiation Oncol Biol Phys 8:903–908, 1982.
91. Vogl SE, Pagano M, Kaplan BH, *et al.*: Cis Platin based combination chemotherapy for advanced ovarian cancer. Cancer 51:2024–2030, 1983.
92. Israel L, Aguilera J, Breau JL: Treatment of advanced ovarian cancer with cis-dichlorodiammineplatinum in combination with cyclophosphamide. Am J Clin Oncol 6:85–89, 1983.
93. Wernz JC, Speyer JL, Noumoff J, *et al.*: Cisplatin/cytoxan: a high dose DDP regimen for advanced stage ovarian carcinoma. Proc Am Soc Clin Oncol 1:112, 1982 (abstract).
94. Young JA, Kroener JF, Lucas WE, *et al.*: Cisplatin, adriamycin, and 5-fluorouracil for advanced ovary cancer. Proc Am Soc Clin Oncol 1:115, 1982 (abstract).
95. Budd GT, Livingston RB, Webster R, *et al.*: Treatment of advanced ovarian cancer with cisplatin, adriamycin and cytoxan (PAC). Proc Am Soc Clin Oncol 1:117, 1982 (abstract).
96. Chung I, Schulman P, Budman D, *et al.*: Phase II trial of hexamethylmelamine, cyclophosphamide, adriamycin and cis-platin combination chemotherapy in advanced ovarian carcinoma. Proc Amer Soc Clin Oncol 1:122, 1982 (abstract).
97. Hernandez E, Rosenshein NB, Villar J, *et al.*: Alternating multiagent chemotherapy for advanced ovarian cancer. J Surg Oncol 22:87–91, 1983.
98. Razis DV, Poulatou E, Petounis A, *et al.*: Treatment of ovarian cancer with a combination of cis-platinum, adriamycin, cyclophosphamide and hexamethylmelamine. Oncology

39:205–208, 1982.
99. Young RC, Howser DM, Myers CE, *et al.*: Combination chemotherapy (Chex-UP) with intraperitoneal maintenance in advanced ovarian adenocarcinoma. Proc Am Soc Clin Oncol 22:465, 1981.
100. Greco FA, Julian CG, Richardson RL, *et al.*: Advanced ovarian cancer: brief intensive combination chemotherapy and second-look operation. Obstet Gynecol 58:199–205, 1981.
101. Greco FA, Burnett LS, Wolff SN, *et al.*: Limited residual advanced ovarian cancer – A curable neoplasm? Proc Am Soc Clin Oncol 1:106, 1982.
102. Williams CJ, Mead GM, Green JA, *et al.*: A randomized study of cisplatin, adriamycin and cyclophosphamide (PACe) versus chlorambucil in stage II and IV ovarian carcinoma. Proc Am Soc Clin Oncol 2:156, 1983 (abstract).
103. Decker DG, Fleming TR, Malkasian GD, *et al.*: Cyclophosphamide plus cis-platinum in combination: treatment program for stage III of IV ovarian carcinoma. Obstet Gyencol 60:481–487, 1982.
104. Carmo-Periera J, Costa FO, Henriques E: cis-Platinum, adriamycin, and hexamethylmelamine versus cyclophosphamide in advanced ovarian carcinoma. Cancer Chemother Pharmacol 10:100–103, 1983.
105. Sturgeon JFG, Fine S, Gospodarowicz MK, *et al.*: A randomized trial of melphalan alone versus combination chemotherapy in advanced ovarian cancer. Proc Am Soc Clin Oncol 1:108, 1982 (abstract).
106. Vogl SE, Kaplan BH, Pagano M: Diamminedichloroplatinum-based combination chemotherapy is superior to melphalan for advanced ovarian cancer when age is 50 and tumor diameter 2 cm. Proc Am Soc Clin Oncol 1:119, 1982 (abstract).
107. Bell DR, Woods RL, Levi JA, *et al.*: Advanced oarian cancer: a prospective randomized trial of chlorambucil versus combined cyclophosphamide and cis-diamminedichloroplatinum. Aust N Z J Med 12:245–249, 1982.
108. Bruckner HW, Cohen CJ, Goldberg JD, *et al.*: Improved chemotherapy for ovarian cancer with cis-diamminedichloroplatinum and adriamycin. Cancer 47:2288–2294, 1981.
109. Neijt JP, tenBokkel Huinink WW, vd Burg MEL, *et al.*: Combination chemotherapy with HEXA-CAF and CHAP-5 in advanced ovarian carcinoma: a randomized study of the Netherlands Joint Study Group for Ovarian Cancer. Proc Amer Soc Clin Oncol 2:148, 1983 (abstract).
110. Imholz G, Mayr AC, Schmoranz W, *et al.*: Cisplatin, adriamycin, cytoxan (PAC) vs fluorouracil, adriamycin, cytoxan (FAC) in ovarian cancer FIGO II B-IV. Preliminary results of a randomized cooperative trial. Proc Amer Soc Clin Oncol 2:155, 1983 (abstract).
111. Omura GA, Ehrlich CE, Blessing JA: A randomized trial of cyclophosphamide plus adriamycin with or without cis-platinum in ovarian carcinoma. Proc Amer Soc Clin Oncol 1:104, 1982 (abstract).
112. Edwards CL, Herson J, Gershenson DM, *et al.*: A prospective randomized clinical trial of melphalan and cis-platinum versus hexamethylmelanime, adriamycin, and cyclophosphamide in advanced ovarian cancer. Gynecol Oncol 15:261–277, 1983 (abstract).
113. Pouillart P, Bretaudeau B, Palangil T, *et al.*: Adenocarcinoma of the ovary (stage III and IV) treated with a combination of adriamycin, cyclophosphamide, 5-fluorouracil and cis-DDP: a study of the therpaeutic role of cis-DDP in the combination. Bull Cancer (Paris) 69:434–442, 1982.
114. Stehman FB, Ehrlich CE, Einhorn LH, *et al.*: Long term follow-up and survival in stage III-IV epithelial ovarian cancer treated with cis-dichlorodiammine platinum, adriamycin and cyclophospamide (PAC). Proc Am Soc Clin Oncol 3:147, 1983 (abstract).
115. Bruckner HW, Cohen CJ, Goldberg J, *et al.*: Ovarian cancer: comparison of adriamycin and cisplatin ± cyclophosphamide. Proc Am Soc Clin Oncol 2:152, 1983 (abstract).
116. Barker CH, Wiltshaw E: Randomized trial comparing low-dose cisplatin and chlorambucil

with low dose cisplatin, chlorambucil, and doxorubicin in advanced ovarian carcinoma. The Lancet 1:747–750, 1981.
117. Bruckner HW, Cohen CJ, Goldberg JD, *et al.*: Cisplatin regimens and improved prognosis of patients with poorly differentiated ovarian cancer. Am J Obstet Gynecol 145:653–658, 1983.
118. Barlow JJ, Piver MS, Lele SB: Weekly cis-platinum remission 'induction' and combination drug consolidation and maintenance in ovarian cancer. Proc Am Soc Clin Oncol 1:119, 1982 (abstract).
119. Vogl SE: Personal communication, 1983.
120. Ozols RF, Garvin AJ, Costa J, Simon RM, Young RC: Histologic grade in advanced ovarian cancer. Cancer Treat Rep 63:255–263, 1979.
121. Grosh WW, Brenner DE, Burnett LS, *et al.*: Dose response and survival in pathologically evaluated patients with ovarian carcinoma treated with brief intensive hexamethylmelamine, cyclophosphamide, doxorubicin and cis-platin (HCAP). Proc Am Assoc Cancer Res 24:145, 1983 (abstract).
122. Alberts DS, Moon TE, Stephens RA, *et al.*: Randomized study of chemoimmunotherapy for advanced ovarian carcinoma: a preliminary report of a Southwest Oncology Group Study. Cancer Treat Rep 63:325–331, 1978.
123. Creasman WT, Gall SA, Blessing JA, *et al.*: Chemoimmunotherapy in the management of primary stage III ovarian cancer: A Gynecologic Oncology Group Study. Cancer Treat Rep 63:319–323, 1979.
124. Calvert AH, Harland SJ, Newell Dr, *et al.*: Early clinical studies with Cis-diammine-1, 1-cyclobutane dicarboxylate platinum II. Cancer Chemother Pharmacol 9:140–147, 1982.
125. Harrap KR, Jones AM, Wilkinson CR, *et al.*: Antitumor, toxic and biochemical properties of cisplatin and eight other platinum complexes. In: Cisplatin: Current status and new developments. Prestayko AW, Crooke ST, Carter SK (ed), New York, Academic Press, 1980, p 193.
126. Jones RB, Collins JM, Myers CE, *et al.*: High volume intraperitoneal chemotherapy with methotrexate in patients with cancer. Cancer Res 41:55–59, 1981.
127. Jones RB, Myers CE, Guarino AM, *et al.*: High volume intraperitoneal chemotherapy ('belly bath') for ovarian cancer. Cancer Chemother Pharmacol 1:161–166, 1978.
128. Howell SE, Pfeifle CE, Wang WE, Olshen RA: Intraperitoneal Cis-diamminedichloroplatinum with systemic thiosulfate protection. Cancer Res 43:1426–1432, 1983.
129. Pfeifle CE, King ME, Howell SB: Pharmacokinetics and clinical efficacy of intraperitoneal cytarabine treatment of advanced ovarian cancer. Proc Am Soc Clin Oncol 2:150, 1983 (abstract).
130. Speyer JL, Collins JM, Dedrick RL, *et al.*: Phase I pharmacological studies of 5-fluorouracil administered intraperitoneally. Cancer Res 40:667–672, 1980.
131. Ozols RE, Young RC, Speyer JL, *et al.*: Intraperitoneal adriamycin in ovarian carcinoma. Proc Am Assoc Cancer Res 21:425, 1980 (abstract).
132. Markman M, Green MR, Pfeifle CE, *et al.*: Combination intracavitary chemotherapy in patients with stage III-IV ovarian carcinoma failing standard treatment regimens. Proc Am Soc Clin Oncol 2:147, 1983 (abstract).
133. Omura G: Personal communication, 1983.

9. Intraperitoneal chemotherapy for ovarian carcinoma

MAURIE MARKMAN and STEPHEN B. HOWELL

A. Introduction

The first step in killing a tumor cell with a chemotherapeutic agent is to deliver the drug to the cell. Whether the cell is intrinsically sensitive or realtively resistant, the effect of the drug is proportional to the product of its concentration and the duration of exposure. In the case of tumors largely confined to extravascular cavities, such as ovarian carcinoma, there is a legitimate concern that injection of cytotoxic agents intravenously may not be the optimal way to get drug to the tumor. Following intravenous injection, most drugs have ready access to the well vascularized marrow and gut, but because of longer diffusional distances they may have much less access to intraperitoneal tumor. This is analogous to the problem of delivering drug to brain tumors that reside at least partially behind the blood-brain barrier [1]. A number of investigators have tried to improve drug delivery to the tumor by instilling cytotoxic agents directly into the peritoneal cavity, but early studies were done in a limited number of patients using small injection volumes and the results were not particularly exciting [2-4]. However, during the past five years there has been a resurgence of interest in using the intraperitoneal (i.p.) route, spurred initially by work performed at the National Cancer Institute which provided a solid pharmacologic rationale for this approach. It is now apparent that, with the exception of hexamethylmelamine, all of the drugs with major activity against ovarian carcinoma can be given safely by the i.p. route, and that this results in a total drug exposure that is 1–3 orders of magnitudes greater than that for plasma. In this chapter, we will outline the pharmacologic principles of i.p. chemotherapy, review both older and recent clinical trials, and point out the theoretical and practical limitations of this approach to improving the therapy of ovarian carcinoma.

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B. Pharmacologic basis for intraperitoneal chemotherapy

1. *Relative clearances and first pass metabolism*

In its simplest form, the body of a patient with ovarian carcinoma can be viewed as consisting of two compartments, the first being the peritoneal cavity containing the tumor, and the second being the rest of the body. Drug placed into the peritoneal cavity will diffuse out into the systemic circulation, and the rate at which its concentration falls will be a function of the volume (V) in the cavity, the surface area available for diffusion (A), the permeability of this surface (P), and the difference in free drug concentration between the peritoneum (C_{pe}%) and the plasma (C_{pl}) [5]. Mathematically the product of permeability times area (PA) is equivalent to the peritoneal clearance, and this relationship is quantitatively defined by Equation 1.

$$\frac{dC_{pe}}{dt} = \frac{PA(C_{pe} - C_{pl})}{V_{pe}} \qquad \text{(Equation 1)}$$

If all of the drug diffusing out of the peritoneum reaches the systemic compartment, then the concentration in the plasma is determined by the volume of this compartment (V_D), the rate at which drug is entering it [$PA(C_{pe} - C_{pl})$], and the rate at which drug is being cleared from the plasma (kC_{pl}) as given by Equation 2.

$$\frac{dC_{pl}}{dt} = \frac{PA(C_{pe} - C_{pl}) - kC_{pl}}{V_D} \qquad \text{(Equation 2)}$$

Equations 1 and 2 can be integrated to yield Equation 3 [5], which indicates that if the concentration in the peritoneal cavity is maintained at a constant level long enough for the system to come into steady-state, then the ratio of the concentration in the peritoneum to that in the plasma is inversely related to the clearances of the two compartments.

$$\frac{C_{pe}}{C_{pl}} = \frac{Cl_{pl}}{Cl_{pe}} + 1 \qquad \text{(Equation 3)}$$

Stated another way, the more difficulty a drug has in getting out of the peritoneal cavity (low peritoneal clearance), and the more promptly the drug is removed once it reaches the systemic compartment (high plasma clearance), the higher the concentration ratio. Since drug exposure is the product of concentration and time (area under the concentration times time curve, AUC), a large difference in clearance will also result in a large difference in total drug exposure. Anything that decreases peritoneal clearance or enhances systemic clearance will have the effect of further augmenting the AUC ratio.

While this model is overly simplistic, it serves both to point out the critical role of clearance as a determinant of drug exposure, and also to highlight the importance of drug metabolism during transit from the peritoneum to the plasma. Currently available data [6–8] suggests that drugs in the molecular weight range of most cancer chemotherapeutic agents are removed from the peritoneal cavity predominantly via the portal circulation. Metabolism of a drug to an inactive form during its first pass through the liver will have the effect of reducing the amount of drug reaching the systemic circulation, and thus further augment the difference in total drug exposure for the two compartments.

Using this type of modelling, Dedrick and his colleagues at the National Cancer Institute [5] calculated the expected peritoneal and plasma concentrations following i.p. administration of methotrexate (MTX). They noted that peritoneal clearance for many drugs (PA) is roughly inversely proportional to molecular weight to the -0.67 power, yielding an estimate of 8 ml/min for MTX. With a systemic clearance of 131 ml/min, their modelling suggested that i.p. administration could potentially result in a 25-fold greater exposure for the peritoneal cavity than for the plasma. In the case of cytarabine, a drug with a large plasma clearance and extensive first pass metabolism by hepatic cytidine deaminase [9], modelling suggested that concentration differences between the peritoneum and plasma would be in the range of 1000-fold. Subsequent pharmacokinetic studies of both of these drugs [10–12] demonstrated that the predictions made by this modelling were remarkedly accurate.

2. Route of absorption

Pharmacokinetic studies give information on the relative total drug exposure for the peritoneal cavity and the plasma, but they do not indicate exactly how drug is absorbed from the cavity. There are potentially three routes by which drugs instilled into the peritoneal cavity can reach the systemic circulation. A drug may be absorbed into the portal circulation and pass through the liver, it may be absorbed into capillaries of the parietal peritoneum that drain directly into the systemic venous circulation, or it may enter the peritoneal lymphatics and reach the venous circulation via the thoracic duct. Relatively little actual data is available for any drug, but several considerations suggest that the great majority of drugs in the molecular weight range of most cancer chemotherapeutic agents are absorbed via the portal circulation. In dogs Kraft *et al.* [6] found that radioactive sodium sulfate appeared in the portal vein within seconds, whereas the concentration in the inferior vena cava was initially many fold less, and appearance of radiolabel in thoracic duct lymph required several minutes. This general

pattern of absorption was confirmed by Lukas *et al.* [7] in rats for atropine, caffeine, glucose, glycine, and progesterone. This is consistent with data indicating that 91% of the effective peritoneal perfusion in rats originates from the splanchnic circulation [13]. It is also consistent with anatomic studies indicating that those structures that make up the greatest part of the surface area, the visceral peritoneum, the omentum and the mesentary, drain into the portal circulation, whereas only the parietal peritoneum drains directly into the systemic circulation [14, 15]. There is a rich network of lymphatics on the undersurface of the diaphragms that communicate directly with the peritoneal cavity via specialized pores [16]. However, the flow in the lymphatic channels is so much less than that in the portal circulation [17, 18] that quantitatively absorption via lymphatics is probably not significant except for compounds of molecular weight 1000 and above [19].

5-Fluorouracil is the only chemotherapeutic agent for which a direct attempt has been made to assess the route of absorption. Speyer *et al.*al. [8] placed portal vein catheters in 4 patients receiving i.p. 5-fluorouracil, and calculated that 29–100% of the drug was absorbed via the portal circulation. However, these values are at best only estimates since the contribution of portal flow to total hepatic flow could not be measured with certainty.

3. Determinants of peritoneal drug clearance

In order to enter capillaries of the portal or systemic circulation, an intraperitoneally instilled drug must cross the single layer of flattened cells that constitutes the peritoneal membrane proper, diffuse through a tissue space, and finally across the capillary membrane [20]. A good deal of information has been gathered on the transport of metabolic wastes, drugs, and toxins from the blood to the peritoneal cavity during peritoneal dialysis [21–25], but much less information is available on the absorption of drugs from the peritoneal cavity into the bloodstream. One of the major unanswered questions relevant to transport in either direction is the extent to which transport is limited by a diffusion barrier versus inadequate delivery of drug to, or removal from, the peritoneal membrane by blood flow [20].

Torres *et al.* [19] performed a systematic study of the importance of molecular weight, lipid-water partition coefficient, association constant (pKa), and instilled volume on the rate of absorption from the peritoneal cavity in rats. They found that fluid absorption was most rapid during the first hour following instillation of enough isotonic fluid (50 ml) to fully distend the peritoneal cavity, and that the rate subsequently slowed. The percent absorbed during the first hour was greater with the instillation of small volumes (10 ml) than with large volumes (75 ml), but the absolute volume

absorbed was greatest for the largest amount instilled. The peritoneal cavity was able to buffer 50 ml volumes from pH 3 to 11 and return the solution to neutral within 1 hour. This observation is consistant with the data of Holcenberg *et al.* [26] who found that after washing the peritoneal cavity, concentrations of small molecules such as amino acids were fully restored within 6 hours, indicating rapid transit into the cavity.

Torres *et al.* [19] found that absorption of water soluble compounds diminished approximately 5-fold as the molecular weight was increased from 18 to 1000, a range that encompasses the molecular weights of all of the drugs with established activity against ovarian carcinoma. Water soluble drugs were more slowly absorbed than lipid soluble drugs, with a drug with a heptane:water solubility ratio of 0.001 having approximately 30% slower absorption than a drug having a solubility ratio of 0.05. The absorption of unionized drugs was found to be faster than that of ionized drugs. Over the pKa range of 2.8 to 9.9 absorption increased approximately 6-fold for a series of acids, and over a pKa range of 0.9 to 9.6 it decreased approximately 2.5-fold for a series of bases. In summary, absorption from the peritoneum appeared to be consistent with passive diffusion restricted by a lipid membrane barrier.

Data are now available from human pharmacokinetic studies on the peritoneal clearance of most of the drugs with established activity against ovarian carcinoma (Table 1). For 5-fluorouracil, cytarabine, melphalan, and methotrexate, the peritoneal clearances cluster between approximately 6.6 and 15 ml/min/m^2. Although its molecular weight is similar to several other drugs in the table, the peritoneal clearance of cisplatin is exceptionally high. This is probably accounted for by the fact that cisplatin is cleared from the peritoneum not only by diffusion out of the cavity, but also by reaction with nucleophilic sites on proteins and small molecules such as cysteine in the peritoneal fluid [27–29]. Over the limited range of observed values, there is not a very good correlation between peritoneal clearance and either molecular weight or the heptane:water solubility ratio. This is not altogether unexpected, however, since each drug differs in both parameters as well as in pKa.

4. Drug distribution

Characteristically ovarian carcinoma seeds the whole of the peritoneal cavity and the rich lymphatic network on the undersurfaces of the diaphragms [39]. If i.p. chemotherapy is to be effective, then drug containing fluid must be distributed to all involved areas. It has often been assumed in the past that since the peritoneal cavity is potentially free flowing, drugs instilled in small volumes (3–500 ml) will be well distributed into all parts of

Table 1. Chemical characteristics and pharmacokinetics of drug active against ovarian carcinoma administered by the intraperitoneal route

Drug	Molecular weight	Hepatana: water solubility ratio[a]	Plasma clearance (ml/min/m^2)	Peritoneal clearance (ml/min/m^2)	Mean peak peritoneal/plasma concentration ratio	Mean peritoneal/plasma AUC ratio	References
5-fluorouracil	130	0.09	2200[b]	14[c]	298	367	8, 30 31
Cytarabine	243	0.005	673	15	664	474	12, 32
Cisplatin	300	0.001	172[d]	43	20	12	33
Melphalan	323	0.1	194	14	93	65	34, 35
Methotrexate	454	0.001	120	6.6	92	NR[e]	10
			114	4.8	NR[e]	NR[e]	11
Doxorubicin	544	NR[e]	514	NR[e]	474	NR[e]	36, 37

[a] Data from reference 38.
[b] At an infusion rate of 1.5 g/m^2/day.
[c] Ml/min.
[d] Howell, S.B., unpublished data.
[e] NR, not reported.

the cavity. However, there is now good documentation from several studies that this is not the case [40–43], and the need for a large enough volume of drug-containing fluid to distend the cavity has become a major principle of i.p. therapy.

Rosenshein *et al.* [43] studied the distribution of ^{99}Tc labelled serum albumin following instillation into the peritoneal cavity of monkeys in volumes equivalent to 0.2% (10 ml) or 5% (250 ml) of their body weight. Following the small volume instillation, the radioactivity was limited to only a portion of the cavity, and neither vigorous massaging of the abdomen nor head up or head down tilting of the animal improved the distribution. In contrast the large volume instillation resulted in uniformly good distribution of radioactivity throughout the abdomen, and head up and head down tilting produced a shift of the whole distribution toward the diaphragms or toward the pelvis respectively.

In addition to poor i.p. distribution, there would appear to be an intrinsic danger associated with injection of large amounts of drug in small volumes. This may potentially result in extremely high concentration pockets of drug capable of producing focal bowel necrosis or other serious toxicity. This situation would completely defeat all of the pharmacologic advantage to be gained by the i.p. route of administration.

Large volumes may assure good distribution of drug in the normal peritoneal cavity, but patients with ovarian carcinoma often have adhesions that may limit the free flow of fluid. We have studied the distribution of ^{99}Tc-sulfur colloid instilled in 2 liters of normal saline as part of a phase I study of i.p. cisplatin at the UCSD Cancer Center [33]. Among 10 ovarian carcinoma patients with far advanced disease all of whom had gone through at least one laparotomy, the ^{99}Tc-sulfur colloid was distributed into all four quadrants in 7 when scanned immediately after instillation. Dunnick *et al.* [44] examined the distribution of i.p. Hypaque instilled in a large enough volume to distend the cavity in 9 patients with ovarian carcinoma and one with peritoneal melanoma. Distribution was complete and unimpaired in 8 of the 10 patients when examined by computerized axial tomography. Using the same technique at the UCSD Cancer Center, we have noted that, even in patients in whom the initial distribution is not complete, there is gradual appearance of contrast material in much of the remaining portions of the peritoneal space over the period of several hours. Thus, the existing data suggests that despite the common occurrence of adhesions, when large volumes are used to distend the peritoneal cavity, drug distribution is adequate in the majority of patients, and even in patients with limited distribution there is often slow exchange of drug into other portions of the cavity. The ability to instill extremely high drug concentrations and maintain these concentrations for long periods of time should favor movement of drug into slowly exchanging areas of the cavity, and indeed excellent clinical re-

sponses have been observed even in patients with very poor initial ^{99}Tc-sulfur colloid distribution [33].

5. *Tumor penetration*

As the modelling suggested [5], pharmacokinetic studies (Table 1) have now demonstrated that the i.p. route of administration results in a very large increase in total exposure for the peritoneal cavity for most of the drugs known to be active against ovarian carcinoma. However, total drug exposure for the peritoneal cavity is not the same as total drug exposure for tumor in the peritoneal cavity. Ovarian carcinoma cells can exist either free floating in the cavity, or as part of nodules growing on the peritoneal surface, or buried in the omentum or masses of fibrous adhesions. Some parts of the tumor are primarily dependent on diffusion from a free surface for delivery of nutrients, whereas other portions are dependent on capillary flow, and this may vary enormously from patient to patient. At the present time there is no information on what fraction of a human ovarian carcinoma is dependent on free surface diffusion versus capillary flow, or what fraction of the tumor resides within a defined distance from a free surface.

Ideally one would like to maximize the delivery of drug by both capillary flow and free surface diffusion. In general cancer chemotherapeutic agents have poor tissue diffusibility [1], and one concern regarding the use of i.p. therapy is that, for a significant portion of the tumor, free surface diffusion might not make up for a potential decrease in capillary drug delivery. It is now apparent that this is not a problem for methotrexate, 5-fluorouracil, cisplatin, melphalan, and cytarabine [10–12, 30, 33, 35]. In the case of each of these drugs, the i.p. dose can be escalated to the point where the amount of drug leaking into the systemic circulation is equivalent to that which can be delivered by a maximum tolerated dose injected intravenously before local toxicity in the peritoneal cavity is encountered. In the case of doxorubicin, peritonitis is the dose limiting toxicity when administered by the i.p. route, and this precludes the attainment of maximum tolerated systemic drug exposure [37]. It is intuitively apparent that for free floating tumor cells and very small nodules, i.p. instillation of drug is likely to result in greater total drug exposure than the intravenous route. However, it is important to emphasize that for all of the drugs other than doxorubicin, used at maximum tolerated doses, even large tumor masses will receive increased drug exposure if any fraction of the tumor has a free surface exposed to the drug-containing fluid. Thus the major issue for methotrexate, 5-flourouracil, cisplatin, melphalan, and cytarabine is how much more total drug exposure can really be achieved with the i.p. route.

In general, penetration of chemotherapeutic agents into tumor spheroids in culture is not good [45, 46]. There are no studies in which actual measurements of tissue penetration have been made with human ovarian carcinoma. By virtue of its intrinsic fluorescence, Ozols *et al.* [47] were able to monitor the penetration of doxorubicin into the murine M5076 model tumor growing intraperitoneally. Following i.p. doxing, staining was observed in only the outermost 6 cell layers. However, the relationship between cellular staining and cytotoxicity is uncertain, and despite the apparent limited penetration, i.p. doxorubicin was curative for this tumor at doses that were ineffective when administered intravenously.

Several groups have modelled the tissue penetrance of drugs, and these models can be used to make predictions about the effects of changes in one or more of the parameters that control drug exposure [20, 48–57]. Table 2 lists some of the factors important in controlling total drug exposure for a tumor nodule growing on the peritoneal surface.

In theory, intraperitoneally administered drug can enter the tumor nodule without having to cross a limiting membrane. However, even when a pseudomembrane is present it is usually only one or several cell layers thick and probably offers little in the way of resistance to diffusion. As the drug diffuses along the extracellular channels between tumor cells, it is at risk for: a) being metabolized to an inactive form in the extracellular fluid; b) being taken up into cells where it can be bound or metabolized; and c) diffusing into capillaries or lymphatics whose drug concentration may be very much lower than that in the extracellular fluid. Each one of these will have the effect of reducing the amount of drug available to diffuse further into the nodule. If the concentration of drug in the peritoneal cavity is held constant, then eventually the profile of decreasing drug concentration as a function of distance into the nodule will reach steady-state. Dedrick *et al.* [20] have proposed a distributed model for the penetration of drugs from the periton-

Table 2. Factors controlling total drug exposure due to free surface diffusion

1. Tumor geometry and surface area
2. Surface drug concentration
3. Plasma drug concentration
4. Duration of exposure
5. Diffusion coefficient of the drug
6. Fraction of the tumor volume which is extracellular fluid
7. Rate of cellular uptake of the drug
8. Intracellular/extracellular drug ratio
9. Drug half-life in extra-cellular fluid
10. Drug half-life in the tumor cell
11. Capillary permeability and area/gram of tumor
12. Capillary flow/gram tumor.

eal surface which is similar in essence to models proposed by Fenstermacher *et al.* [48] and Levin *et al.* [1] for the diffusion of drugs in brain. In the absence of significant metabolism or tissue binding, the gradient of drug concentration as a function of distance into the tumor is given by Equation 4, where C_x is the concentration at distance x

$$\frac{C_x}{C_{pe}} = e^{-\sqrt{\frac{pa}{D}} \cdot X} \qquad \text{(Equation 4)}$$

from the surface, C_{pe} is the peritoneal concentration, p is the capillary permeability, a is the capillary area, and D is the diffusion coefficient for the drug in tumor [20]. Although neither p, a, or D are known for ovarian carcinoma, several useful predictions can be made from this model [20].

1. The tissue gradient will be exponential and dependent on the product of capillary permeability and area, and diffusivity. The greater the diffusivity of the drug and the lower its rate of uptake into capillaries, the greater the penetration. The depth at which the concentration is 37% of that at the tumor surface is given by $\sqrt{D/pa}$.
2. The depth of penetration will not be very sensitive to the molecular weight of the drug. This follows from observations that in a variety of tissues, capillary permeability is proportional to the -0.63 power of the molecular weight, and the diffusion coefficient (in water) to the -0.45 power, so that pa/D varies only with the -0.09 power of molecular weight. As molecular weight increases, capillary permeability falls more rapidly than diffusivity, thus all other drug characteristics being equal, large drugs will penetrate further than small drugs.
3. The time required for the tissue profile to reach steady-state will vary inversely with capillary permeability, diffusivity, and rate of metabolism. Until steady-state is reached, the duration of exposure to intraperitoneally instilled drug will be an important determinant of the depth of penetration.

Collins *et al.* [51, 52] estimated the tissue penetration of 5-fluorouracil using this model and data obtained from peritoneal instillations in the rat. Their data were consistent with the following predicted behavior of the concentration profile:

1. The time required to reach a steady-state tissue profile will be a function of the peritoneal drug concentration. The higher the concentration the longer the time required.
2. The slope of the profile can be expected to be flatter at high i.p. concentrations. With an instilled concentration of 12 mM, tissue concentration will drop to 5% of the surface concentration at a depth of more

than 600 uM into the tissue, whereas at a surface concentration of 24 uM, the tissue concentration will reach 5% of the peritoneal level at a depth of less than 200 uM. This reflects the varying contribution of metabolism to the removal of 5-fluorouracil; at high concentrations metabolism is saturated and capillary uptake becomes relatively more important. The effect of saturable metabolism is to steepen the concentration profile as the drug penetrates deeper.

The only experimental confirmation of the predictions made by this model comes from measurements of the penetration of drugs from the cerebrospinal fluid into the caudate nucleus of the brain [48–50]. Blasberg *et al.* [49] found that the brain concentration had fallen to 1% of the cerebrospinal fluid concentration after 1 hour of exposure at approximately 1.2 mm for BCNU, 1.4 mm for thiotepa, 1.8 mm for cytarabine, 2.2 mm for methotrexate, and 3.1 mm for hydroxyurea. In the case of thiotepa the tissue prolife was already at steady-state after 15 minutes of exposure, whereas hydroxyurea was not at steady-state at 1 hour. Further modelling of the behavior of methotrexate [50] suggested that its profile was still not at steady-state even after 24 hours of exposure, and that using a surface concentration of 1 mM, cytotoxic concentrations of at least 1 uM would extent at least 15 mm into the brain.

The above modelling data are presented here in some detail: 1) because they offer a plausible explanation for the relative lack of toxicity of intraperitoneally instilled drugs to organs in the abdominal cavity; 2) because they suggest some limitations on the potential curability of i.p. therapy; and 3) because they at the same time suggest ways in which pharmacologic manipulations may further improve the prospects for i.p. treatment. One fo the interesting observations in all of the phase I trials of i.p. therapy published to date is the relative lack of damage to the mucosa of the gut, even when concentrations of drug are used that produce dose-limiting systemic toxicity. The drug-sensitive crypt cells of the gastrointestinal mucosa reside only approximately 1 mm from the peritoneal surface [20], but a very extensive capillary network lies between the mucosa and the serosa. Presumably this network acts like a sink drawing off drug diffusing in from the peritoneal cavity, and this protects the gut. The pre-eminence of capillary uptake as a determinant of drug penetration may serve not only to prevent damage to this normal tissue, but also to facilitate the diffusion of drugs into tumor masses which are realtively less well perfused. Free surface diffusion is unlikely to kill a large fraction of the tumor in cases of advanced disease where large masses are present. However, if drugs are used at near maximum tolerated doses, then the combination of drug delivery by capillary flow and free surface diffusion may still be more effective than intravenous dosing alone. The delivery of drug by capillary flow will significantly flatten

the tissue concentration profile, resulting in the presence of cytotoxic concentrations at deeper levels, and repeated cycles of therapy may result in sequential layers of tumor being destroyed. By using higher molecular weight drugs, clearance from the peritoneal cavity may be decreased and simultaneously tissue penetrance may be improved [51]. The challenge will be to find larger drugs whose systemic clearance does not diminish along with their peritoneal clearance. Finally, pharmacologic approaches to diminish splanchnic blood flow may be effective in reducing not only the peritoneal clearance, but also the rate of removal of drug from tumor masses.

6. Neutralizing agents

The enormous concentration differences that can be maintained between the peritoneal cavity and the plasma (Table 1) present the opportunity to make concurrent use of the antagonists that can specifically block the toxicity of drug leaking into the systemic circulation to further enhance the therapeutic index of i.p. therapy [5]. There are both potential advantages and disadvantages to this approach that depend importantly on the pharmacokinetic and pharmacodynamic characteristics of the agonist/antagonist pair.

Ideally one would like to work with an antagonist that was completely excluded from the peritoneal cavity, and did not diffuse from capillaries into tumor. Practically, however, once the antagonist is injected intravenously it will begin diffusing from the plasma into the peritoneal cavity at a rate which is determined by it concentration and diffusional characteristics, and which may also be influenced by peritoneal and splanchnic blood flow. If the concentration of the antagonist in the plasma is held constant, then in the absence of metabolism or removal of the antagonist from the peritoneal cavity by some other mechanism, the concentration in the cavity will eventually approach that of the plasma. This will occur rapidly for drugs with large peritoneal clearances, and will take progressively longer for drugs with smaller clearances. Likewise, the larger the volume in the peritoneal cavity the longer it will take to reach steady-state. The extent of equilibration will also be influenced by the half-life of the antagonist in the plasma. If an intermittent dosing schedule is used and the plasma half-life is short, then plasma concentration may begin declining before full equilibration has occurred. These same considerations pertain to the delivery of antagonist from plasma to tumor nodules via capillary flow.

Since an antagonist present in the plasma cannot be totally excluded from the peritoneal cavity or tumor, the competitive versus non-competitive nature of the interaction between the agonist and antagonist, and the concentration dependence of the antagonism become important. Selection of a

non-competitive antagonist which could reverse the cytotoxicity of the agonist irrespective of the latter's concentration would seem less wise than the selection of a competitive antagonist. In addition one would like to choose a competitive antagonist whose effectiveness was rapidly lost as the concentration of the agonist was increased over a small concentration range. Thus, if just enough antagonist were introduced into the systemic circulation to neutralize the agonist, then even if the antagonist equilibrated completely into the peritoneal cavity a relatively small increase in the peritoneal concentration of the agonist relative to that in plasma would be sufficient to prevent compromise of the cytotoxicity in the peritoneal cavity. Even in this circumstance, however, introduction of sufficient neutralizing agent into the systemic compartment to prevent marrow or gut toxicity will also compromise the activity of agonist delivered to the tumor by capillary flow, and will result in greater dependence on free surface diffusion if i.p. therapy is to achieve a greater total drug delivery to the tumor. The greater the concentration gradient of the agonist that can be maintained between the peritoneal cavity and the plasma, the less likely this is to be a problem; likewise, the problem will diminish in proportion to the extent that there is selective neutralization of the drug in normal as opposed to malignant tissues.

An alternative strategy for enhancing the therapeutic index of i.p. therapy is to select a neutralizing agent which acts only in the normal tissue manifesting the dose-limiting toxicity of the cytotoxic agent, but which is relatively ineffective at neutralizing the agonist in the general systemic circulation.

At the UCSD Cancer Center we have explored the use of intravenous leucovorin as a neutralizing agent for i.p. methotrexate, and intravenous sodium thiosulfate as a neutralizing agent for intraperitoneally administered cisplatin [10, 33, 53]. Figure 1 shows the ability of various concentrations of leucovorin to protect freshedly aspirated human marrow cells against inhibition of thymidylate synthetase activity by two concentrations of methotrexate, 1 and 10 uM. Leucovorin at 1 uM afforded a small degree of protection against 1 uM methotrexate, but no protection against 10 uM methotrexate. Since only a small proportion of normal marrow thymidylate synthetase activity may be required for marrow proliferation *in vivo*, it was reasoned that 1 uM reduced folate would be just sufficient to protect against the cytotoxicity of 1 uM methotrexate *in vivo*, whereas this concentration of reduced folate would be unable to protect against the toxicity of a 10-fold higher concentration of methotrexate if such a difference could be maintained between the peritoneal cavity and plasma. Figure 2 shows the ability of thiosulfate to protect a human lymphoblast cell line (WI-L2) against the antiproliferative effect of cisplatin. At a cisplatin concentration of 1 uM growth rate was inhibited to less than 20% of control; the addition of progressively higher concentrations of thiosulfate to the culture reversed this

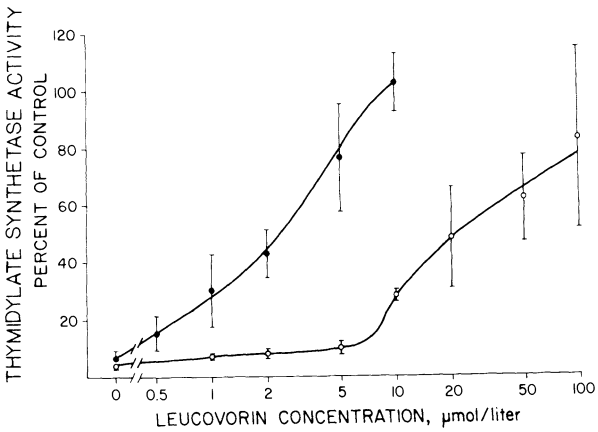

Figure 1. Protection by leucovorin of human bone marrow monocuclear cells against methotrexate-induced inhibition of ^{3}H-deoxyuridine incorporation. Cells were incubated with either 1 uM (●) or 10 uM (○) methotrexate for a hour, and then pulsed with ^{3}H-deoxyuridine for another hour, Mean ±SD. Republished by permission.

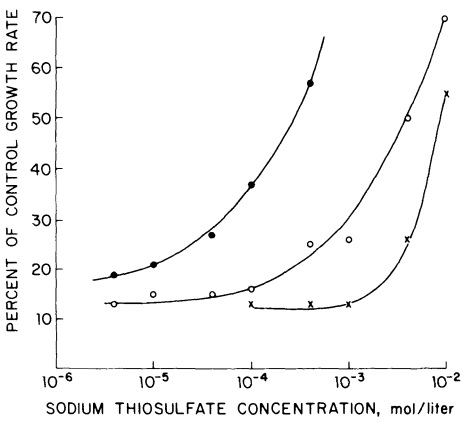

Figure 2. Dose-response curves fort for the antagonism by sodium thiosulfate of cisplatin-induced decrease in the growth rate of WI-L2 cells *in vitro.* Cisplatin 1 uM (●9, 4 uM (○), and 10 uM (×). Antagonism was quantitated by growth rate. Each point represents the mean of triplicate cultures. Republished by permission.

effect. Increasing the cisplatin concentration to 4 and 10 uM shifted the curves to the right. Molar thiosulfate/cisplatin ratios of 280 to 950 were required to restore the growth rate of these representative non-malignant cells to 50% of control, indicating that thiosulfate was not a very potent antagonist of cisplatin. However, the data in Figure 2 suggest that whereas a thiosulfate concentration of 1 mM would protect against the toxicity of 1 uM cisplatin, this concentration of thiosulfate would be ineffective against a 10-fold higher concentration of cisplatin. Thus both leucovorin and thiosulfate appear to be antagonists that fulfill the criteria of a steep concentra-

tion-dependent competitive antagonism of their respective cytotoxic agent. The results of phase I trials of each of these pairs, are discussed in detail in the next section.

C. Single agent clinical trials of intraperitoneal chemotherapy

1. Early trials

In 1955, Weisberger *et al.* reported the use of nitrogen mustard administered i.p. to six patients with ovarian cancer and malignant ascites [2]. Three patients were said to have no reaccumulation of fluid with two additional patients demonstrating a partial response to the drug. While the authors speculated that high local drug concentrations achieved by i.p. drug administration resulted in direct tumor kill, it is more likely to decrease in fluid accumulation was due to a non-specific sclerosing effect of nitrogen mustard. That significant pains was experienced by patients treated by this route supports this argument. In addition, patients also experienced nausea, vomiting and abdominal cramps. These side-effects severely limited the usefulness of nitrogen mustard as an agent to be administered i.p.

Hemisulfur mustard was similarly tried as a therapeutic agent in ovarian cancer. As with nitrogen mustard, six of eleven (55%) of patients treated i.p. with hemisulfur mustard experienced benefit with decreased reaccumulation of malignant ascites. Unfortunately, there was no evidence of shrinkage of any mass lesion, and the only patients benefiting from therapy developed an ileus secondary to chemical peritonitis [3].

An additional alkylating agent, thiotepa, has also been administered i.p. to patients with ovarian cancer [4]. In this study, thiotepa was instilled into the abdominal cavity both at the time to surgery and daily thereafter until white blood cell count depression was noted. Several patients with inoperable or recurrent tumor also received the drug by this route. No direct benefit from the administration of thiotepa i.p. was observed.

Bleomycin has been used i.p. more extensively than the previously mentioned agents [54]. Its principal utility has been in the control of malignant ascites. In one report of 23 patients with ovarian cancer and malignant ascites, 22% experienced a complete disappearance of fluid while an additional 17% had a partial response when 30–180 mg of bleomycin was instilled in a small volume of fluid (100 ml) following drainage of ascites [54]. Twenty-one percent of patients experienced abdominal pain, and fever was also a common side effect [54]. In a second study, one of nine patients experienced benefit from the i.p. administration of bleomycin with decrease in malignant ascitic fluid accumulation [55]. Six of nine patients in

this study experienced fever but abdominal pain was not a significant problem. While the efficacy of bleomycin has been limited to the control of malignant ascites, it remains an interesting drug for i.p. therapy as it is relatively non-myelosuppressive, which makes it of potential value in combination regimens, and it has demonstrated a significant pharmacokinetic advantage when administered by the i.p. route. For the same dose of drug administered intravenously or i.p., the plasma concentration versus time curve is significantly lower for the i.p. route of administration [55]. In addition, the systemic absorption of the drug from the peritoneal cavity has been shown to average only 45% of the administered dose [55, 56].

Two patients have been treated with vinblastine (7 mg/m^2) i.p. in a small volume as part of a phase 1 trial [55]. The drug proved to be toxic with both patients developing an adynamic ileus within 24 hours of drug administration. One patient required an exploratory laparotomy secondary to bowel obstruction. Neither patient responded to treatment with this agent.

2. Phase 1 and pharmacokinetic studies of large volume instillation

Since the modeling study of Dedrick *et al.* [5] was published in 1978, phase 1 and pharmacokinetic trials have been performed using dialysis techniques for all of the major drugs with activity against ovarian carcinoma except for hexamethylmelamine.

a. *Methotrexate*

Methotrexate has been examined in several pilot studies of i.p. drug administration and has demonstrated a significant pharmacokinetic advantage when delivered by this route [10, 11, 38, 57]. In a series of studies conducted at the National Cancer Institute, patients received a 48-hour dialysis with methotrexate (15 to 50 uM) weekly for six weeks. Dialysis fluid was changed every six hours. The treatment volume was initially two liters but was gradually increased to patient tolerance. In addition, patients received intravenous folinic acid rescue (3.5 mg/kg/hr) as a continuous infusion from 40 hours to 56 hours after the initiation of methotrexate instillation. In four patients with ovarian cancer methotrexate concentrations in the peritoneum could be maintained 18–36 times higher than the corresponding values in the plasma. No definite therapeutic benefit was observed in this trial, but toxicity was mild and included myelosuppression, diarrhea, nausea and vomiting, transient liver function abnormalities, and both chemical and bacterial peritonitis [11].

The simultaneous intracavitary administration of methotrexate and intravenous infusion of leucovorin has been evaluated in a pilot study at the UCSD Cancer Center [10]. In this trial, patients with effusions or ascites

received methotrexate at 30 mg/m^2 per day as a constant infusion at 10 ml/hr. The duration of treatment was escalated from six to 120 hours. Leucovorin was simultaneously administered intravenously at 15 mg/m^2 every four hours in an effort to neutralize methotrexate escaping into the systemic circulation. Side effects of this treatment program included mild abdominal pain and myelosuppression (principally thrombocytopenia) with infusions of greater than 96 hours. Mucositis, nephrotoxicity, vomiting or liver function abnormalities were not observed. The single patient with ovarian cancer treated i.p. on this protocol had complete disappearance of ascites for ten months. Thymidylate synthetase activity was inhibited 86% in malignant cells in the effusions while bone marrow cells exhibited only a 46% inhibition. In addition, cytokinetic monitoring of marrow showed no significant perturbation [10]. The four patients receiving i.p. therapy on this clinical trial had a mean steady-state effusion methotrexate concentration of 24.2 uM and an average peritoneal-to-plasma ratio of 92. This study not only confirmed the ability to deliver significantly higher levels of methotrexate to tumors confined to third spaces, but also demonstrated the utility of concurrent neutralizing agents in reducing systemic toxicity.

The first of these two studies of methotrexate demonstrated the safety and pharmacological advantage of the i.p. route, but the total duration of exposure had be limited to 48 hours because of the presence of toxic concentrations of methotrexate in the plasma. In this study leucovorin was used in a rescue mode to rapidly terminate the methotrexate-induced inhibition of DNA in the marrow and gut. However, methotrexate is a cell cycle phase specific agent whose cytotoxicity is a function of both concentration and duration of exposure. During a period of 48 hours it is unlikely that a very large fraction of cells in a slow growing ovarian carcinoma will have passed through the sensitive S phase of the cell cycle. Thus the second study, which used leucovorin in a neutralizing rather than rescue mode, aimed at extending the total duration of exposure for the peritoneal cavity. The latter study established the feasibility of maintaining exposure for five days with acceptable systemic toxicity, but it remains to be proven that the additional cell kill expected from this approach makes up for the expected antagonism of methotrexate delivered to the tumor via capillary flow because of the administration of intravenous leucovorin.

b. *5-fluorouracil*

The i.p. administration of 5-fluorouracil has undergone extensive evaluation at the National Cancer Institute [8, 30, 57]. Ten patients, including five with ovarian cancer, have been treated with one of two treatment regimens: (a) eight consecutive instillations of two liters of drug for four hours each (36 hours total per course), or (b) once per day instillation of two liters of fluid for three to five days [30]. Therapy was repeated every two weeks. Patients

were treated with concentrations ranging from 5 uM to 8 mM (1.3 to 2080 mg/2 liters) 5-fluorouracil. Dose limiting toxicity in these trials included pancytopenia and mucositis at 5-fluorouracil concentrations of 4.5 to 5 mM. In addition, abdominal pain and two documented cases of bacterial peritonitis developed in the patients treated during this study. Two previously treated patients with ovarian cancer had an objective response to therapy including one patient who was found to be free of disease at restaging laparatomy. While a mean of 82% of the administered drug was absorbed in four hours (with i.p. levels declining by first-order kinetics), the average four hour peritoneal fluid concentration was 298 times the simultaneously measured plasma levels. The NCI investigators concluded that for some period of time following i.p. drug administration, tumor in the peritoneal cavity could be bathed with 5-fluorouracil at a concentration one log greater than can be safely achieved by the intravenous administration of this agent. In a companion study, the same investigators demonstrated that the total delivery of 5-fluorouracil to the liver via the portal circulation following i.p. therapy was comparable to that reported following direct intra-arterial administration of this agent [8]. Future clinical trials directly comparing i.p. and intra-arterial drug instillation are required before any statement can be made regarding the relative toxicity and efficacy of these two approaches to treatment of liver metastasis.

c. *Doxorubicin*

The i.p. administration of doxorubicin has been investigated both in humans [37] and in a mouse transplantable ovarian cancer model [47, 58]. As previously discussed, the ability to measure doxorubicin-specific intranuclear fluorescence has allowed an analysis of drug levels in various body tissues of the mouse as well as an estimate of the depth of tissue penetration of this agent [59, 60]. Peak doxorubicin levels in tumor cells were 50 times higher after an i.p. dose compared to the same intravenous dose of the drug. On-the-other-hand, doxorubicin levels in the heart, liver and kidney were significantly higher following intravenous treatment [47]. As anticipated due to its low diffusibility with i.p. drug administration [1], bright doxorubicin fluorescence was demonstrated only in the outermost four to six cell layers of the tumor. Following intravenous treatment faint doxorubicin-specific fluorescence was seen in a patchy distribution throughout the tumor [47].

The efficacy of i.p. doxorubicin in this mouse ovarian cancer system was quite dramatic. Seventy percent of mice receiving a single i.p. 10% lethal dose of doxorubicin (5 mg/kg) demonstrated long term survival (>60 days) when inoculated with 10^6 tumor cells compared to no long term survivors among mice inoculated intravenously with a comparable 10% lethal dose of this drug [58].

In a phase 1 trial conducted at the National Cancer Institute, ten patients with ovarian cancer who had failed systemic chemotherapy were treated with from 9 to 54 uM doxorubicin (10–50 mg/2 liter treatment volume) i.p. as a single four hour dwell administered every two weeks [37]. None of the patients had received prior intravenous doxorubicin. There were five clinical responses, including objective evidence of tumor regression in three patients and significant decrease in ascites in an additional two individuals. The dose limiting toxicity was the development of significant abdominal pain which occured along with ascites and adhesions at doxorubicin concentrations greater than 36 uM (40 mg/2 liters). However, the experience of this group with dosages less than 36 uM was limited (total of 8 courses), and it is possible that if a larger number of courses had been administered at lower dose levels greater local toxicity might have been observed. Peritoneal irritation did not appear to worsen greatly when repeated courses of therapy were administered during this trial. Myelosuppression was mild with only a single course being complicated by a nadir white blood cell count of less than 2000/cu mm.

There was a significant pharmacokinetic advantage for the i.p. administration of doxorubicin demonstrated in this study. While 85% of the drug was absorbed during the four hour dwell time, the mean peritoneal level during this period was 166 times higher than the corresponding plasma level and the peak peritoneal concentration to plasma concentration ratio was 474. In addition, peak plasma levels following the maximally administered i.p. dose of doxorubicin (54 uM, 60 mg/2 liters) were one-tenth that of a 60 mg intravenous dose of the drug [37].

d. *Cisplatin*

At the UCSD Cancer Center we have demonstrated the safety and efficacy of the i.p. instillation of extremely high doses of cisplatin along with intravenously administered sodium thiosulfate used as a neutralizing agent for the cisplatin which enters the systemic circulation [16, 17]. Sodium thiosulfate, an agent used clinically in high doses in man for cyanide poisoning, was demonstrated in a mouse model to significantly protect against cisplatin induced nephrotoxicity. Thisulfate presumably combines with and inactivates the reactive site on the drug, neutralizing both its toxic and anti-tumor effects [61]. While some loss of activity of cisplatin woud be expected to result from the simultaneous administration of sodium thiosulfate, this negative factor was predicted to be far outweighed by the ability to deliver extremely high doses of the drug i.p. [53].

In a recently reported phase 1 trial of i.p. cisplatin, we have administered up to 270 mg/m^2 in a two liter treatment volume as a four hour dwell (with simultaneous intravenous sodium thiosulfate) without evidence of nephrotoxicity [33]. In addition, there was no evidence of significant local toxicity

due to cisplatin as evaluated both by lack of abdominal pain and direct observation (laparoscopy, autopsy) of serosal surfaces following cisplatin therapy. Myelosuppression was mild even at the highest dose levels. There was no evidence of peripheral neuropathy or hearing loss during the trial. Dose-limiting toxicity was significant nausea and vomiting that was only moderately well controlled even with extremely intensive anti-emetic regimens. This observation would imply that significant cisplatin was reaching and remained in the systemic circulation in spite of sodium thiosulfate administration. In fact, while the peak peritoneal concentration of free reactive cisplatin averaged 21-fold higher than the peak plasma level and the area under the peritoneal cisplatin elimination curve averaged 12-fold more than the area under the plasma curve, the area under the concentration curve for the plasma (with an i.p. dose of 270 mg/m^2) *increased* two-fold compared to that reported for native cisplatin after an intravenous dose of 100 mg/m^2 [33]. It is our current hypothesis that while the sodium thiosulfate does indeed neutralize cisplatin, this reaction is slow at the levels of thiosulfate circulating in the plasma, but proceeds rapidly in the kidney where the drug is concentrated. Thus, thiosulfate is an example of a quasi-organ specific neutralizing agent. At the end of the four hour dwell, only 7% of the instilled cisplatin could be recovered from the fluid remaining in the peritoneal cavity. The dose of cisplatin did not influence its peritoneal clearance, nor did clearance change with serial courses of treatment.

Eleven patients with ovarian cancer previously treated with systemic chemotherapy were entered onto this trial. Seven of these patients were evaluable for response to therapy of whom one had an almost complete response of extensive nodular intraperitoneal disease documented by laparoscopy [33]. A second report of the use of lower doses of i.p. cisplatin without sodium thiosulfate has confirmed both the safety and efficacy of this agent in ovarian cancer [62].

e. *Cytarabine*

Cytarabine (cytosine arabinoside), the most active chemotherapeutic agent in acute non-lymphocytic leukemia, has demonstrated limited usefullness against solid tumors [63]. Unfortunately, while the most rational way to administer this cell-cycle phase-specific agent would be to exposure tumor for prolonged periods of time, thus allowing more slowly dividing cells to enter into cycle, toxicity to bone marrow with such therapy would be severe. For several reasons, cytarabine is potentially an ideal drug for i.p. administration. First, as the agent is rapidly inactivated by deamination in the liver [64] and the absorption of compounds administered i.p. is principally through the portal circulation [7], a significant pharmacokinetic advantage for the i.p. administration of this agent would be predicted [5]. Second, it is possible that tumor cells which are relatively resistant to cyta-

rabine in the concentrations achievable through the intravenous administration of this agent, might be quite sensitive at drug concentrations achievable in the peritoneal cavity by direct i.p. administration.

In a recently completed phase 1 trial of the i.p. administration of cytarabine in patients with ovarian cancer refractory to standard forms of chemotherapy, we have confirmed the theoretical advantage of i.p. administration of this agent (Table 1) [12, 65]. Pharmacokinetic evaluation of three patients receiving escalating doses of cytarabine during three consecutive five hour i.p. drug infusions, demonstrated a two to three log difference between peritoneal and plasma concentrations. Total peritoneal drug exposure was 300 to 1000 times greater than that of the plasma when cytarabine was administered by the i.p. route. Peritoneal cytarabine levels demonstrated first-order kinetics with half-lives of 70–210 minutes [65]. In addition, of nine individual tumors tested in an *in vitro* clonogenic assay, three were found to be sensitive to cytarabine only at concentrations greater than that achievable by systemic drug administration, but definitely achievable (for at least some period of time) during i.p. administration of the agent.

Ten patients with refractory ovarian cancer were subsequently treated with courses consisting of 20 consecutive dialysate exchanges (five hour dwell, 1 hour drainage per exchange) over five days with 30 mg of cytarabine (60 uM) in each two liter exchange [12]. Treatment was repeated at 28 day intervals. Myelosuppression was mild with a white blood cell nadir of <2000/cu mm developing during only two of the 25 treatment cycles. Thrombocytopenia (platelet count $<75{,}000$) developed during only one cycle. There was no evidence of chemical peritonitis in any patient. There were nine episodes of bacterial peritonitis in five patients. In all cases of bacterial peritonitis symptoms of low grade fever and mild abdominal tenderness developed. All nine infectious episodes responded rapidly to intraperitoneal and systemic antibiotics. It is likely that the frequent catheter manipulation required as part of this treatment program resulted in the high incidence of infection observed. Two patients had objective responses to therapy with positive cytologies becoming negative for five-plus and twelve-plus months [12]. In one of these patients, follow-up laparoscopic examination has been negative on two occasions.

f. *Melphalan*

Two groups have investigated the use of melphalan administered by the i.p. route. It has been shown *in vitro* that glutamine is an active inhibitor of melphalan uptake by human ovarian carcinoma cells [66]. Holcenberg *et al.* have attempted to increase the activity of this agent when administered i.p. by infusing *Acinetobacter* glutaminase-asparaginase (AGA) shortly before melphalan is instilled into the abdominal cavity [67]. Three previously treated patients with ovarian cancer received from one to four courses of

melphalan plus AGA. The dose of melphalan ranged from nine to 40 mg/m^2. The treatment volume was approximately two liters with the abdomen being drained 24 hours after AGA administration. Measured plasma melphalan was less than six percent of the peritoneal concentration [67]. Toxicity during this trial included fever, nausea and vomiting, abdominal pain, and a single case of ileus that lasted several days. There was no evidence of significant bone marrow suppression, or renal or hepatic dysfunction. One patient responded to therapy with disappearance of ascites after two courses. Laparoscopy did not reveal evidence of residual tumor but one month later a malignant pleural effusion developed.

A phase 1 trial of i.p. melphalan has recently been completed at the UCSD Cancer Center. Thirteen patients have received melphalan administered i.p. at from 30 to 90 mg/m^2 in two liters of normal saline given as a four hour dwell. A significant pharmacokinetic advantage for the i.p. route has been demonstrated with mean peritoneal and plasma areas under the concentration versus time curve at 60 mg/m^2 being 52.1 and 1.42 ug hr/ml, respectively. The geometric mean ratio of area under the concentration versus time curve for peritoneum to plasma for all courses was 65. Dose-limiting toxicity was bone marrow suppression at the 70 mg/m^2 dose level. A single patient with gastric cancer had objective evidence of an anti-tumor effect. Unfortunately, only one of the heavily pretreated patients with ovarian cancer responded to this treatment program with conversion to negative cytology [12].

g. *Mitomycin*

In a preliminary report, the i.p. administration of mitomycin C has been investigated in 5 patients [68]. Five to 30 mg of this agent were delivered in 1.5 liters of fluid. Mitomycin C was undetectable in the plasma except at the 30 mg dose. A 200 fold pharmacokinetic advantage for the i.p. administration of this agent was demonstrated. There was no myelosuppression observed but two patients developed evidence of a chemical peritonitis at the 30 mg dose level.

h. *Misonidazole and demethylmisonidazole*

The i.p. administration of the hypoxic radiosensitizers misonidazole and demethylmisonidazole has been investigated in a phase 1 trial in six patients with advanced ovarian cancer [69]. Drugs were administered in two liters of fluid and drained after remaining in the peritoneal cavity three hours. Patients received concomitant whole abdominal irradiation as part of their treatment program. Three hours after the radiosensitizer administration, the concentration of drug in the peritoneal fluid was more than eight times that of plasma concentrations. The clearance of misonidazole from the peritoneum was 19.1 ml/min while for demethylmisonidazole this value was

12.4 ml/min. The AUC for the peritoneal cavity was 3.2 times greater than the plasma AUC for misonidazole and 7.6 times greater for demethylmisonidazole. Toxicity in this trial included nausea, vomiting, diarrhea, abdominal pain, and a single episode of mild paresthesias. This approach to the use of radiosensitizers has significant appeal both as a method of reducing the dose-dependent neurotoxicity of these agents when administered systemically, and for increasing local drug concentration in intraperitoneally localized tumors during radiation therapy. In addition, these agents are synergistic *in vitro* with certain chemotherapeutic agents, including cisplatin, and may have a role in combination intracavitary chemotherapy [70].

D. Combination chemotherapy trials

There is significant theoretical rationale as well as clinical experience to support the use of combination intraperitoneal chemotherapy for ovarian cancer as well as other malignancies confined to body cavities. Goldie and Coltman [71] have presented a mathematical model of the development of tumor cell resistance to chemotherapeutic agents that strongly suggests that mutations confering such resistance occur during tumor growth, and that the best possible way to prevent this from occuring would be to use multiple agents early in the treatment program. This model further supports the use of alternating, non-cross resistant drug combinations as a method to expose tumors to as many drugs as possible in an effort to prevent drug resistance to a single agent [72].

Additional support for the use of combination intraperitoneal chemotherapy in ovarian cancer comes from clinical trials which demonstrate a signifcantly increased overall and complete response rate with the use of combination intravenous chemotherapy [73]. Unfortunately, while the response rates are higher with combination regimens, the majority of patients with ovarian cancer will ultimately relapse and die of their disease.

Cisplatin has been shown to be an extremely active agent in ovarian cancer with complete responses rates in previously untreated patients receiving cisplatin-based regimens reported to be as high as 60% [74–76]. Our previously described experience with i.p. cisplatin administered as a single-agent has demonstrated that this agent can be given in extremely high doses (with sodium thiosulfate protection of the kidneys) with acceptable toxicity [33]. It is, therefore, an ideal agent upon which to build a combination i.p. drug regimen.

We have also demonstrated that cytarabine can safely be administered by the i.p. route with a significant pharmacokinetic advantage over intravenous drug administration. In a series of *in vitro* experiments investigating cisplatin synergy with a variety of chemotherapeutic agents, marked synergy was

demonstrated between cisplatin and cytarabine against LoVo cells (a human colon carcinoma cell line) [77–79]. While cytarabine was totally inactive against this cell line when administered alone, it markedly enhanced cell kill when added in culture with cisplatin. This effect was concentration dependent with a remarkable 1,600-fold increased cell kill being observed at a cytarabine concentration of 4×10^{-2} M [79].

Doxorubicin was selected along with the cisplatin and cytarabine for inclusion in our initial combination i.p. chemotherapy regimen. As previously mentioned, the National Cancer Institute had reported that this agent could be administered i.p. with acceptable local and systemic toxicities [37]. In addition, several reports of cisplatin and doxorubicin administered together intravenously have demonstrated this combination to be among the most active against ovarian cancer [75, 76].

Following initial *in vitro* evaluation of the stability of the three drug mixture in solution, we initiated a phase 1 trial of escalating dosages of the drug combination in patients with ovarian cancer failing standard chemotherapy regimens, or in individuals with other malignancies principally confined to the abdominal cavity. At the present time, this study is the only reported trial of combination i.p. chemotherapy for advanced intraabdominal malignancies [80]. The three drugs to be administered were mixed together in two liters of normal saline and infused i.p. with a dwell time of four hours. In addition to overnight hydration, sodium thiosulfate was simultaneously infused starting at the time of i.p. instillation with a bolus (4 grams/m^2) and followed by a constant infusion (12 grams/m^2/over 6 hours).

During the trial the dose of cisplatin was escalated from 100 mg/m^2 to 200 mg/m^2 while the dose of cytarabine was increased from 49 mg (10^{-4} M) to 490 mg (10^{-3} M). The initial dose of doxorubicin (20 mg/2L) proved to cause excessive local abdominal discomfort with 60% of courses at this dose level being associated with pain lasting greater than 72 hours or requiring narcotic analgesia. The dose of doxorubicin was subsequently reduced to 2 mg/2L with a marked decrease in the amount of abdominal pain. Unfortunately, even at this dose level 10–20% of courses were still associated with a significant amount of pain.

Nephrotoxicity during this trial was mild with only eight percent of courses (4 of 48) administered at the 200 mg/m^2 dose level of cisplatin being associated with serum creatinine rises to ≥ 2.0 (normal value in our laboratory: ≤ 1.5). There was no evidence of nephrotoxicity in 37 courses administered at the 100 mg/m^2 dose level of cisplatin. Nephrotoxicity only developed in patients heavily pretreated with cisplatin and serum creatinines returned to baseline in all patients within four weeks of therapy.

Myelosuppression was also mild with only three of 99 courses of therapy being complicated by white blood cell depression to less than 2000/cu mm. Similarly, thrombocytopenia (platelet nadir $<$75,000/cu mm) developed

during only three courses. Hospitalization was not required by any patient for complications of pancytopenia. In addition, in all but one course where bone marrow suppression was observed, patients had received the 20 mg dose of doxorubicin.

Unfortunately, our success in controlling cisplatin-induced emesis, even with intensive antiemetic regimens, has been limited. However, no patient has been forced to discontinue therapy because of this problem. There were no other significant toxicities observed during this trial.

Seven of 19 evaluable patients had objective evidence of tumor response to treatment. Twelve patients were not evaluable for response as they had disease defined at exploratory surgery which was not easily followable by physical examination or laboratory evaluation. Five of 14 patients with ovarian cancer responded to treatment with significant and often dramatic decrease in malignant ascites lasting from three to nine-plus months. In addition, one patient with a large vaginal mass had a decrease in the size of the mass lesion. Three patients (two with ovarian cancer) had conversion of positive cytologies to negative lasting from three to eight-plus months following the initiation of therapy. One patient with ovarian cancer remains in a clinical complete remission eight months after beginning treatment. The single patient with adenocarcinoma-of-unknown-primary had a decrease in ascites, weight gain of 20+ pounds, and disappearance of signs of partial small bowel obstruction (>30 bowel movements/day, abdominal pain) which continues at seven-plus months following the initiation of i.p. chemotherapy.

In an effort to take further advantage of the remarkable synergy reported between cisplatin and cytarabine, we have recently instituted a phase 1–2 trial of i.p. cisplatin and cytarabine with the later agent administered at a significantly escalated dose level. To date, 19 patients (15 with ovarian cancer) failing standard forms of chemotherapy for their disease have received 32 courses of cisplatin at 100 mg/m^2 or 200 mg/m^2 and cytarabine at 2000 mg (4×10^{-3} M) or 4500 mg (10^{-2} M).

A most interesting toxicity was observed in the two patients who received a total of three courses of this treatment program with the cisplatin being given at 200 mg/m^2 and cytarabine at 10^{-2} M (4500 mg). While significant myelosuppression (WBC count <2000/cu mm) did not occur during any of the three courses, thrombocytopenia was observed following treatment in each course with nadir counts of 57,000, 15,000, and 13,000/cu mm. One would have expected that if the bone marrow toxicity were due to the effects of cytarabine alone both the white blood cells as well as the platelets would have been affected. When cisplatin causes bone marrow depression it is generally the platelets that are depressed. However, in our phase 1 trial of i.p. cisplatin administered as a single agent, or in our combination regimen discussed in this section, doses of cisplatin comparable to that administered

in the current trial were given without the development of thrombocytopenia. Thus, it would appear that cytarabine is acting synergistically with cisplatin in depressing platelet production. This clinical experience supports the *in vitro* observation of a profound synergistic action of cytarabine on cisplatin-induced cytotoxicity [77–79].

In the 29 courses of therapy where cytarabine was administered at a dose of 2000 mg (4×10^{-3} M), only a single course each was associated with thrombocytopenia (nadir count of 71,000/cu mm) or significant myelosuppression (WBC nadir 600/cu mm). In addition, only one course of therapy was associated with a serum creatinine rise to ≥ 2 mg % (4%). Doxorubicin was not included in the present treatment regimen specifically to avoid the problem of abdominal pain, particularly with repeated courses of therapy. In this preliminary evaluation local abdominal discomfort following i.p. therapy with cisplatin and cytarabine appears to be less than that observed with the inclusion of doxorubicin (three of 32 courses being associated with pain lasting more than 72 hours).

To date, nine of the 19 patients are evaluable for response to therapy. Five patients have had definite objective responses including three patients with ovarian cancer. One patient had developed evidence of hydronephrosis requiring a ureteral stint to be placed following two cycles of intravenous cisplatin, doxorubicin and cyclophosphamide. A CT scan of the abdomen after two cycles of i.p. chemotherapy demonstrated significant shrinkage of retroperitoneal adenopathy. A second patient had no response to melphalan and had developed large bilateral pleural effusions and massive ascites requiring drainage every two to three days. Following her first cycle of therapy she had a dramatic decrease in the rate of fluid accumulation in her abdomen and no reaccumulation of fluid in her chest with a one month follow-up. A third patient with positive peritoneal fluid cytologies prior to therapy has had negative cytologies for two plus months. In addition, two patients with adenocarcinoma-of-unknown-primary with disease in the abdomen have had evidence of response to therapy. One patient with massive intra-abdominal disease, ascites and evidence of almost complete bowel obstruction at the time of surgery, is presently completely asymptomatic, gaining weight and demonstrates almost complete disappearance of ascites after three cycles of treatment.

E. Overview

1. Disadvantages of intraperitoneal chemotherapy

There are both practical and theoretical disadvantages to the i.p. route for the delivery of chemotherapeutic agents relative to the intravenous method

of drug administration. The most important practical problem is that insertion of a catheter, whether temporary or semi-permanent (Tenckhoff catheter), is required. Therefore, there is the added risk to the patient of bowel perforation (particularly with blind insertion of a percutaneous catheter in a patient with adhesions but without ascites), anaesthesia (with surgical placement of the catheter), and infection. Infection can potentially be introduced both at the time of catheter placement and when the catheter is manipulated to administer chemotherapy or drain the abdomen, although with one exception [12] the incidence of infection in the phase 1 trials reported to date has been low. This may be related to the fact that several of the agents are reasonably good antibiotics. The introduction of totally implantable ports that can be connected to a Tenckhoff catheter (e.g. Port-a-cath^{Tm}) and placed subcutaneously on the abdominal wall has resulted in a much better patient acceptance, and promises to reduce the incidence of infection.

A second practical problem is that of catheter failure. Catheter failure in the UCSD Cancer Center experience is rarely due to plugging of the catheter with clot or debris, but rather is due to the formation of a fibrous tunnel around the catheter which causes it to function like a one way valve. These catheters accept fluid well, and often good distribution within the abdomen is maintained, but they do not drain. This problem will require some means of preventing adhesion formation within the abdomen. In our recent experience we would estimate that 10–20% of catheters will eventually develop this problem.

The major theoretical disadvantage of intraperitoneal chemotherapy is the fact that there are parts of the tumor to which drug is better delivered by capillary flow than by diffusion from the peritoneal cavity. It appears very likely that the intraperitoneal route can increase total drug delivery to free floating tumor cells, and small tumor nodules growing on the accessible surface of the peritoneum, but there is legitimate concern that this approach may not be effective for larger tumor masses. However, it is now apparent that for 5-fluorouracil, methotrexate, cisplatin, cytarabine, and melphalan, the i.p. dose can be escalated to the point where just as much drug reaches the systemic circuit as could be delivered by the intravenous route before local toxicity in the peritoneal cavity is encountered. Thus, if the extraordinarily high concentrations present in the peritoneal cavity contribute any additional drug exposure over and above that due to capillary flow, then for these drugs there should be a therapeutic advantage for the intraperitoneal route even for larger tumors. The validity of this hypothesis remains to be tested in larger clinical trials.

2. Advantages of intraperitoneal chemotherapy

The major reason for selecting the i.p. over the intravenous route for administration of chemotherapeutic agents is the potential of markedly increasing drug delivery to at least a portion of the tumor. The phase 1 and pharmacokinetic studies completed to date have demonstrated that all of the major drugs with activity against ovarian carcinoma (with the exception of hexamethylmelamine which has not been investigated) can be safely administered by the i.p. route. For all of these drugs the peak concentration achievable in the peritoneal cavity far exceeds that attainable in the plasma, and total drug exposure for the cavity is 1 to 3 orders of magnitude greater than for the plasma.

The number of drugs with activity against ovarian carcinoma at high concentrations may be much larger than the small number of drugs with clinically useful activity at the concentrations achievable in the plasma, and thus the i.p. route may enlarge the range of useful drugs for development of non-cross resistant treatment programs. The finding that cytarabine is active against ovarian carcinoma at concentrations 10 uM or more is one example where the i.p. route of administration permits the use of a drug that would not otherwise be chosen for the treatment of ovarian carcinoma. The feasibility of using very high concentrations may also permit clinicians to make better use of concentration-dependent synergy, such as that between cytarabine and cisplatin [77–79].

The ability to maintain cytotoxic concentrations of cell-cycle phase-specific agents in contact with the tumor for very long periods of time, while at the same time only minimally exposing the systemic circulation creates, for the first time, the opportunity to rationally use this group of drugs against ovarian carcinoma. It has already been demonstrated that methotrexate and cytarabine can be maintained at concentrations well above those necessary for the killing of most cells *in vitro* for periods of up to 5 days with relatively little systemic toxicity. By reducing peritoneal concentrations somewhat, it may be possible to maintain continuous drug exposure for a month or more.

Another possible advantage of the i.p. route is its potential for delivery of large amounts of drug to the liver for the treatment of primary tumor or secondary metastases. Since most hepatic tumors are thought to receive their major blood flow from the hepatic artery rather than the portal circulation, it remains to be proven that increased drug delivery via the i.p. route will result in a therapeutic benefit. The i.p. route may also result in delivery of very high concentrations into the diaphragmatic and mediastinal lymphatics and thus be of use for the treatment of tumors involving these channels as well.

A fourth major potential advantage of the i.p. route is that, for the first

time, it may allow the effective differential synchronization of tumor and marrow. It is clear that synchronization of a tumor cell population followed by application of an appropriately timed second dose of drug can markedly increase total cell kill [81].

Finally, the potential advantages of the i.p. route also pertain to the intra-pleural route of drug administration. The direct instillation of repeated doses of chemotherapeutic agents into the pleural space with the intention of eliminating tumor rather than sclerosing the cavity may permit better control of mesothelioma and other tumors confined to this extravascular space.

3. Future directions

Treatment of human tumors by the i.p. route remains an experimental method of drug delivery. While the previous section has outlined the major theoretical advantages for the administration of chemotherapy via this route for tumors principally confined to the peritoneal cavity (e.g., ovarian cancer), there are several major questions that need to be addressed in attempting to optimally use this treatment modality.

First, there is a major need to develop improved catheters and other techniques for i.p. drug delivery. The development of a new delivery system whereby multiple catheters are placed into the abdominal cavity might improve drug distribution. The delivery of continuous low-dose long-duration i.p. chemotherapy will require the development of a system where catheter failure (development of one-way valve) occurs with less frequency and manipulation of the infusion apparatus is kept at a minimum (decrease risk of infection).

Second, additional information on the direct penetration of specific chemotherapeutic agents into tumor masses needs to be obtained. At present, doxorubicin is the single agent for which even limited knowledge of this important issue is available [47].

Third, drugs not previously believed to have majority activity against ovarian cancer will have to be evaluated for activity when administered in high concentrations by the i.p. route. In particular, drugs with higher molecular weights might be demonstrated to have low peritoneal cavity clearances and yet reasonably good tumor penetration.

Fourth, it will be necessary to explore the clinical utility of long duration exposure of intraperitoneal tumor to cell-cycle phase-specific agents. As mentioned, this will require the development of improved delivery systems.

Fifth, future studies will need to address the question of tumor cytokinetics and the optimal timing of subsequent drug dosing. It might be possible

to deliver intensive i.p. therapy on day 1 and subsequently administer an i.p. dose of a second drug(s) on day 8–10 to take advantage of potential tumor recruitment resulting from the initial cell kill.

Finally, when optimal drug combinations and schedules have been defined, it will be necessary to demonstrate in controlled clinical trials whether the pharmacokinetic advantage of i.p. drug administration can be translated into improved response rates and survival.

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References

1. Levin VA, Patlak CS, Landahl MD: Heuristic modelling of drug delivery to malignant brain tumors. J Pharmacokinetics and Biopharmaceutics 8:257–296, 1980.
2. Weisberger AS, Levine B, Storaasli JP: Use of nitrogen mustard in treatment of serous effusions of neoplastic origin. JAMA 159:1704–1707, 1955.
3. Green TH: Hemisulfer mustard in the palliation of patients with metastatic ovarian carcinoma. Obstet Gynecol 13:383–393, 1959.
4. Kottmeier HL: Treatment of ovarian carcinomas with thiotepa. Clin Obstet Gynecol 11:428–438, 1968.
5. Dedrick RL, Myers CE, Bungay PM,DeVita VT, Jr: Pharmacokinetic rationale for peritoneal drug administration in the treatment of ovarian cancer. Cancer Treatment Rep 62:1–9, 1978.
6. Kraft AR, Tompkins RK, Jesseph JE: Peritoneal electrolyte absorption: analysis of portal, systemic venous and lymphatic transport. Surgery 64:148–153, 1968.
7. Lukas G, Brindle SD, Greengard P: The route of absorption of intraperitoneally administered compounds. J Pharmacol Exp Ther 178:562–566, 1971.
8. Speyer JL, Sugarbaker PH, Collins JM, Decrick RL, Klecker RW Jr, Myers CE: Portal levels and hepatic clearance of 5-fluorouracil after intraperitoneal administration in humans. Cancer Res 41:1916–1922, 1981.
9. Chabner BA, Johns DG, Coleman CN, Drake JC, Evans WH: Purification and properties of cytidine deaminase from normal and leukemic granulocytes. J Clin Invest 53:922–931, 1974.

10. Howell SB, Chu BCF, Wung WE, Metha BM, Mendelsohn J: Long-duration intracavitary infusion of methotrexate with systemic leucovorin protection in patients with malignant effusions. J Clin Invest 67:1161–1170, 1981.
11. Jones RB, Collins JM, Myers CE, Brooks AE, Hubbard SM, Balow JE, Brennan MR, Dedrick RL, DeVita VT: High-volume intraperitoneal chemotherapy with methotrexate in patients with cancer. Cancer Res 41:55–59, 1981.
12. Pfeifle CE, King ME, Howell SB: Pharmacokinetics and clinical efficacy of intraperitoneal cytarabine treatment of advanced ovarin cancer. Proc Am Soc Clin Oncol 2:150, 1983.
13. Bulkley GB: Washout of intraperitoneal xenon: effective peritoneal perfusion as an estimation of splanchnic blood flow. In: Granger DN, Bulkley GB (eds) Measurements of Blood Flow: Applications to the Splanchnic Circulation. Williams and Wilkins, Baltimore, Chapter 24, pp 441–453, 1981.
14. Lockhart RD, Hamilton GF, Fyfe FW: Anatomy of the human body. J.B. Lippincott Company, Philadelphia, 1960.
15. Romanes GB: Editor, Cunningham's Textbook of Anatomy. Oxford University Press, London 1964.
16. Leak LV, Rahil K: Permeability of the diaphragmatic mesothelium: the ultrastructural basis for 'stomata'. Am J Anat 151:577–594, 1978.
17. Bollman JL, Cain JC, Grindlay JH: Techniques for the collection of lymph from the liver, small intestine, or thoracic duct of the rat. J Lab Clin Med 33:1349–1352, 1948.
18. Reininger EJ, Saperstein LA: Effect of digestion on distribution of blood flow in the rat. Science 126:1178–0000, 1957.
19. Torres IJ, Litterst CL, Guarino AM: Transport of model compounds across the peritoneal membrane in the rat. Pharmacology 17:330–340, 1978.
20. Dedrick RL, Flessner MF, Collins JM, Schults JS: Is the Peritoneum a membrane? Am Soc Artificial Int Organs 5:1–8, 1982.
21. Nolph KD, Popovich RP, Ghods AJ, Twardowski: Determinants of low clearances of small solutes during peritoneal dialysis. Kid Int 13:117–123, 1978.
22. Nolph KD: Short dialysis, middle molecules, and uremia. Ann Int Med 86:93–97, 1977.
23. Maher JF: Principles of dialysis and dialysis of drugs. Am J Med 62:475–481, 1977.
24. Nolph KD: Peritoneal clearences. J Lab Clin Med 94:519–525, 1979.
25. Maher JF: Peritoneal transport rates: Mechanisms, limitation and methods for augmentation. Kid Int 18:117–120, 1980.
26. Holcenberg J, Anderson T, Ritch P, Skibba J, Howser D, Ring B, Adams S, Helmswoth M: Intraperitoneal chemotherapy with melphalan plus glutaminase. Cancer Res 43:1381–1388, 1983.
27. Burchenal JH, Kalaher K, Dew K, Lokys L, Gale G: Studies of cross-resistance, synergistic combinations and blocking of activity of platinum derivaties. Biochimie 60:961–965, 1978.
28. LeRoy AF, Lutz RJ, Dedrick RL, Litterst CL, Guarino AM: Pharmacokinetic study of cis-dichlorodiammineplatinum (II) (DDP) in the beagle dog: thermodynamic and kinetic behavior of DDP in a biologic milieu. Cancer Treatment Rep 63:59–70, 1979.
29. Himmelstein KJ, Patton TF, Belt RJ, Taylor S, Repta AJ, Sternson LA: Clinical kinetics of intact cisplatin and some related species. Clin Pharmacol Ther 29:658–664, 1981.
30. Speyer JL, Collins JM, Dedrick RL, Brennan MF, Buckpitt AR, Londer H, DeVita VT Jr, Myers CE: Phase I pharmacological studies of 5-fluorouracil administered intraperitoneally. Cancer Res 40:567–672, 1980.
31. Howell SB, Wung WE, Taetle R, Hussain F, Romine JS: Modulation of 5-fluorouracil toxicity by allopurinol in man. Cancer 48:1281–1289, 1981.
32. Slevin ML, Piall EM, Aherne GW, Johnston A, Lister TA: The pharmacokinetics of cytosine arabinoside in the plasma and cerebrospinal fluid during conventional and high dose therapy. Medical and Pediatric Oncology Supplement 1:157–168, 1982.

33. Howell SB, Pfeifle CE, Wung WE, Olshen RA, Lucas WE, Yon JL, Green M: Intraperitoneal cisplatin with systemic thiosulfate protection. Ann Intern Med 97:845–851, 1982.
34. Alberts DS, Chang SY, Chen H-SG, Moon TE, Evans TL, Furner RL, Himmelstein K, Gross JF: Kinetics of intravenous melphalan. Clin Pharmacol Ther 26:73–80, 1979.
35. Pfeifle CE, Howell SB, Olshen RA, Wung WE: Intraperitoneal melphalan: A phase I study. Am Soc Clin Pharm Therap 33:223, 1983.
36. Greene RF, Collins JM, Jenkins JF, Speyer JL, Myers CE: Plasma pharmacokinetics of adriamycin and adriamycinol: Implications for the design of *in vitro* experiments and treatment protocols. Cancer Research 43:3417–3421, 1983.
37. Ozols RF, Young RC, Speyer JL, Sugarbaker PH, Green R, Jenkins J, Myers CE: Phase I and pharmacological studies of adriamycin administered intraperitonelly to patients with ovarian cancer. Cancer 42:4265–4269, 1982.
38. Jones RB, Myers CE, Guarino AM, Dedrick RL, Hubbard SM, DeVita VT: High volume intraperitoneal chemotherapy ('belly bath') for ovarian cancer. Cancer Chemother Pharmocol 1:161–166, 1978.
39. Katz ME, Schwartz PE, Kapp DS, Luikart S: Epithelial carcinoma of the ovary: current strategies. Ann Int Med 95:98–111, 1981.
40. Tully TE, Goldberg ME, Loken MK: The use of ^{99}mTc-sulfur colloid to assess the distribution of ^{32}P chromic phosphate. J Nucl Med 15:190–191, 1974.
41. Taylor A Jr *et al.*: Loculation as a contraindication to intracacitary ^{32}P chromic phosphate therapy. J Nucl Med 16:318–319, 1975.
42. Vider M, Deland Fm, Maruyama Y: Loculation as a contraindication to intracavitary ^{32}P chromic phosphate therapy. J Nucl Med 17:150–151, 1976.
43. Rosenshein N, Blake D, McIntyre PA, Parmley T, Natarajan TK, Dvornicky J, Nickoloff E: The effect of volume on the distribution of substances instilled into the peritoneal cavity. Gynecologic Oncology 6:106–110, 1978.
44. Dunnick NR, Jones RB, Doppman JL, Speyer J, Myers CE: Intraperitoneal contrast infusion for assessment of intraperitoneal fluid dynamics. AJR 133:221–223, 1979.
45. West GW, Weichselbau R, Little JB: Limited penetration of methotrexate into human osteosarcoma spheroids as a proposed model for solid tumor resistance to adjuvant chemotherapy. Cancer Res 40:3668, 1980.
46. Durand RE: Flow cytometry studies of intracellular adriamycin in multicell spheroids *in vitro*. Cancer Res 41:3495–3498, 1981.
47. Ozols RF, Locker GY, Doroshow JH, Grotzinger KR, Myers CE, Young RC: Pharmacokinetics of adriamycin and tissue penetration in murine ovarian cancer. Cancer Res 39:3209–3214, 1979.
48. Fenstermacher JD, Patlak CS, Blasberg RG: Transport of material between brain extracellular fluid, brain cells and blood. Federation Proceedings 33(9):2070–2074, 1974.
49. Blasberg RG, Patlak C, Fenstermacher JD: Intrathecal chemotherapy: Brain tissue profiles after ventriculocisternal perfusion. J Pharmacology & Experimental Therapeutics 195(1):73–83, 1975.
50. Blasberg RG, Patlak CS, Shapiro WR: Distribution of methotrexate in the cerebrospinal fluid and brain after intraventricular administration. Cancer Treatment Reports 61:633–641, 1977.
51. Collins JM, Dedrick RL, Flessner MF, Guarino AM: Concentration-dependent disappearance of fluorouracil from peritoneal fluid in the rat: Experimental observations and distributed modeling. J Pharma Sci 71(7):735–738, 1982.
52. Collins JM, Dedrick RL, King FG, Speyer JL, Myers CE: Non-linear pharmacokinetic models for 5-fluorouracil in man: intravenous and intraperitoneal routes. Clin Pharmacol Ther pp 235–246, 1980.
53. Howell SB, Pfeifle CE, Wung WE, Olshen RA: Intraperitoneal cisplatin with systemic thiosulfate protection. Cancer Res 43:1426–1431, 1983.

54. Ostrowski MJ, Halsall GM: Intracavitary bleomycin in the management of malignant effusions: A multicenter study. Cancer Treat Rep 66:1903–1907, 1982.
55. Alberts DS, Chen H-SC, Chang SY, Peng YM: The disposition of intraperitoneal bleomycin, melphalan, and vinblastine in cancer patients. Recent Results in Cancer Research 74:293–299, 1980.
56. Alberts DS, Chen H-SG, Mayersohn M, Perrier D, Moon TE, Gross JF: Bleomycin pharmacokinetics in man. Cancer Chemother Pharmacol 2:127–132, 1979.
57. Speyer JL, Myers CE: The use of peritoneal dialysis for delivery of chemotherapy to intraperitoneal malignancies. Recent Results in Cancer Research 74:264–269, 1980.
58. Ozols RF, Grotzinger KR, Fisher RI, Myers CE, Young RC: Kinetic characterization and response to chemotherapy in a transplantable murine ovarian cancer. Cancer Res 39:3202–3208, 1979.
59. Schwartz HS: A fluorometric assay for daunomycin and adriamycin in animal tissues. Biochemical Medicine 7:396–404, 1973..
60. Donelli MG, Martini A, Colombo T, Bossi A, Garattini S: Heart levels of adriamycin in normal and tumor-bearing mice. Europ J Cancer 12:913–923, 1976.
61. Howell SB, Taetle R: Effect of sodium thiosulfate on cis-Dichlorodiammineplatinum (II) toxicity and antitumor activity in L1210 leukemia. Cancer Treat Rep 64(4–5):616–616, 1980.
62. Casper ES, Kelson DP, Alcock NW, Lewis JL, Jr: Ip cisplatin in patients with malignant ascites: Pharmacokinetic evaluation and comparison with the i.v. route. cancer Treat Rep 67(3):235–238, 1983.
63. Wasserman TH, Comis RL, Goldsmith M, *et al.*: Tabular analysis of the clinical chemotherapy of solid tumors. Cancer Chemother Rep 6(3):399–419, 1975.
64. Ho DH, Frei E3rd: Clinical pharmacology of 1-beta-D-arabinofuranosyl cytosine. Clin Pharmacol Ther 12:944–954, 1971.
65. King ME, Pfeifle CE, Markman M, Howell SB: Cytarabine pharmacokinetics following intraperitoneal administration. Clin Pharmacol Ther 33:222, 1983.
66. Vistica DT, Von Hoff DD, Torain B: Uptake of melphalan by human ovarian carcinoma cells and its relationship to the amino acid content of ascitic fluid. Cancer Treat Rep 65(1–2):157–161, 1981.
67. Holcenberg J, Anderson T, Ritch P, Skibba J, Howser D, Ring B, Adams S, Helmsworth M: Intraperitoneal chemotherapy with melphalan plus glutaminase. Cancer Res 43:1381–1388, 1983.
68. Gyves J, Ensminger W, Niederhuber J, Manuzak P, Van Harken D, Janis MA, Stetson P, Knutsen C, Doan K: Phase I study of intraperitoneal (IP) 5 day continuoua 5-FU infusion and bolus mitomycin C. Proc ASCO 1:15, 1982.
69. Gianni L, Jenkins JF, Greene RF, Lichter AS, Myers CE, Collins JM: Pharmacokinetics of the hypoxic radiosensitizers misonidazole and demethylmisonidazole after intraperitoneal administration in humans. Cancer Res 43:913–916, 1983.
70. Stratford IJ, Adams GE, Horsman MR, Kandaiya S, Rajartnam S, Smith E, Williamson C: The interaction of misonidazole with radiation, chemotherapeutic agents, or heat. Cancer Clin Trials 3:231–236, 1980.
71. Goldie JH, Coltman AJ: A mathematic model for relating the drug sensitivity of tumors to their spontaneous mutation rate. Cancer Treat Rep 63:1727–1733, 1979.
72. Goldie JH, Coldman AJ, Gudauskas GA: Rationale for the use of alternating non-cross-resistant chemotherapy. Cancer Treat Rep 66(3):439–449, 1982.
73. Young RC, Chabner BA, Hubbard SP, Fisher RI, Bender RA, Anderson T, Simon RM, Canellos GP, DeVita, VT: Advanced ovarian adenocarcinoma: A prospective clinical trial of melphalan (L-PAM) versus combination chemotherapy. N Engl J Med 299:1261–1266, 1978.
74. Gershenson DM, Wharton JT, Herson J, Edwards CL, Rutledge FN: Single-agent cis-Pla-

tinum therapy for advanced ovarian cancer. Obstet Gynecol 58(4):487–496, 1981.
75. Williams CJ, Mead B, Arnold A, Green J, Buchanan R, Whitehouse M: Chemotherapy of advanced ovarian carcinoma. Cancer 49:1778–1783, 1982.
76. Cohen CJ, Goldberg JD, Holland JF, Bruckner HW, Deppe G, Gusberg SB, Wallach RC, Kabakow B, Rodin J: Improved therapy with cisplatin regimens for patients with ovarian carcinoma (FIGO Stages III and IV) as measured by surgical end-staging (second-look operation). Am J Obstet Gynecol 145:955–967, 1983.
77. Drewinko B, Green Catherine, Loo TL: Combination chemotherapy *in vitro* with cis-dichlorodiammineplatinum (II). Cancer Treat Rep 60(11):1619–1625, 1976.
78. Bergerat J-P, Green C, Drewinko B: Combination chemotherapy *in vitro*. IV. response of human colon carcinoma cells to combinations using cis-diamminedichloroplatinum. Cancer Biochem Biophy 3:173–180, 1979.
79. Bergerat J-P, Drewinko B, Corry P, Barlogie B, Ho DH: Synergistic lethal effect of cis-dichorodiammineplatinum and 1-Beta-D-arabinofuranosylcytosine. Cancer Res 41:25–30, 1981.
80. Markman M, Green MR, Pfeifle CE, Lucas WE, Yon JL, Howell SB: Combination intracavitary chemotherapy in patients with stage III-IV ovarian carcinoma failing standard treatment regimens. Proc ASCO 2:147, 1983.
81. Vaughan WP, Burke PJ: Development of a cell kinetic approach to curative therapy of acute myelocytic leukemia in remission using the cell cycle-specific drug 1-beta-d-arabinofuranosylcytosine in a rat model. Cancer Res 43:2005–2009, 1983.

10. Chemotherapy followed by whole-abdominal radiation therapy for ovarian cancer

A. GOLDHIRSCH, R. GREINER and B. DAVIS

Introduction

Recently, the addition of cis-platinum to combination chemotherapy has resulted in an improved complete response rate as compared to combination regimens which do not include platinum (40% vs. 19%, respectively) [1]. Unfortunately, the reported follow-up of patients treated with platinum-containing combination chemotherapies is generally less than five years [2–6] (Table 1). It is clear from these results that a substantial portion of the complete remitters after combination chemotherapy will fail after three or more years of follow-up. These patients obviously had undetected microscopic disease which was resistant to chemotherapy.

While controversial, radiation does have a role in treatment of selected ovarian cancer patients. In those patients with macroscopic bulk residual disease, radiation therapy offers no benefit [7]. On the other hand, radiation therapy has been demonstrated to be effective in Stage Ib, II, and asymptomatic III presentations with minimal residual disease after a complete primary surgical procedure [8]. In this setting, whole abdominal radiation ('open field' or 'moving strip') [9] is superior to pelvic radiation combined with, or without, single-agent chemotherapy. Moreover, these regimens are most effective in patients with no visible residual disease after surgery. Forty-six patients with Stage III disease who had no visible residual disease after surgery were treated with pelvic and abdomino-pelvic radiotherapy at the Princess Margaret Hospital in Toronto. They were followed from 1971 to 1981 (minimum follow-up was 2 years). All of these patients had a complete hysterectomy and bilateral adnexectomy. The estimated ten-year survival of this population is 48% [10].

It may be gleaned from these data that combination chemotherapy alone, even in the setting of 'second-look'-confirmed complete remission, or radiation therapy alone will not cure the majority of patients with advanced ovarian carcinoma. One may conclude that:

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Table 1. Results of combination chemotherapy for advanced ovarian cancer: complete remission

Ref.	Combination	No. patients	No. CR (%)	Med. duration of CR's (mos.)	Med. survival of CR's (mos.)	No. CR failures	'Long-term' results no. CR ⩾36 mos.
Barker 1981 [2]	DDP/CLB	46	13 (28%)	31+	—	4	4
	DDP/CLB/ADM	39	11 (28%)	24+	—	5	2
Williams 1982 [3]	PACe	35	21 (60%*)	16.6*	26.5*	15?*	2
Young 1978 [4]	HexaCAF	40	13 (33%)	30+	?	4	?
Stehman 1983 [5]	PAC-I PAC-V	56	23 (41%)	33	33	16	7
Greco 1983 [6]	HexaCAP	58	22 (38%)	>36+	>36+	8	14

* Clinically assessed complete response

DDP, P = cis-platinum; CLB = chlorambucil; ADM, A = adriamycin; C = cyclophosphamide; HexaCAF = hexamethylmelamine, cyclophosphamide, methotrexate, 5-fluorouracil.

1. Combination chemotherapy is effective in shrinking bulk disease and inducing complete remissions.
2. Whole abdominal irradiation is effective in eradicating microscopic residual disease.

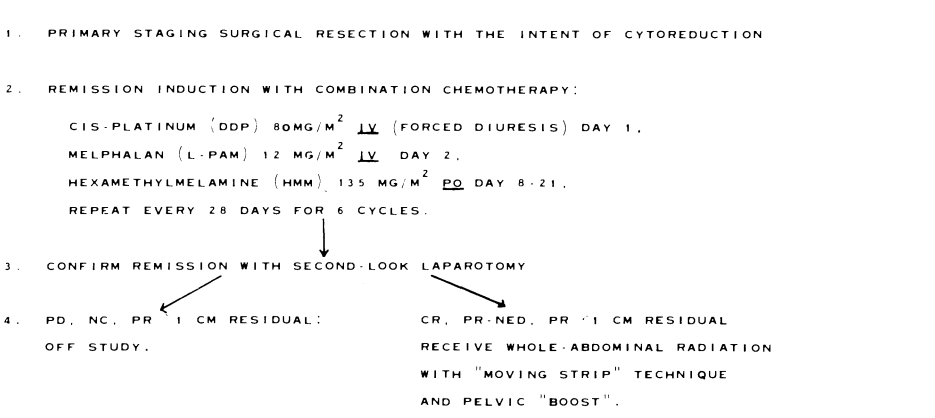
A combined modality approach

We and others have proposed that the solution to this problem of cure is a combination of the therapeutic approaches which are known to be effective, i.e. remission induction and eradication of microscopic residual disease. To test this hypothesis, an aggressive combined modality approach was initiated in 1979 (Table 2) for treatment of the advanced stages of ovarian cancer.

The major tumor cell burden is often contained in the primary tumor masses, and the larger the tumor burden the more likely it is that there will be specific drug-resistant tumor cell subpopulations. When the primary masses are removed by surgery, chemotherapy has a much better chance of further reducing tumor burden since the growth fraction of micrometastases is larger and the probability of specifically drug-resistant populations smaller [11]. Thus, a primary surgical staging procedure with the intent of maximal cytoreduction should be the approach in every patient [12, 13]. If this operation is incomplete, the probability of cure may be reduced.

For the purpose of remission induction a combination chemotherapy is given within one month of surgery. Since the spontaneous development of resistant cells may occur in as little as six population doubling times [14],

Table 2. Combined modality approach to advanced ovarian cancer; the HexaPAMP abdominal radiation protocol



delay in initiating chemotherapy is avoided. For the combination chemotherapy, three agents known to be active in the treatment of ovarian cancer were selected: cis-platinum (DDP), melphalan (L-PAM), and hexamethylmelamine (HMM). Intravenous L-PAM was chosen since its biological availability is more reliable than that of oral administration [15]. The low dose of 12 mg/m^2 of L-PAM was chosen because renal function impairment on the day after DDP administration is frequently observed and the toxicity from L-PAM given in these conditions may be severe [16].

The second-look operation is performed after six cycles of the induction protocol in patients demonstrating an objective clinical response. Remissions may thus be confirmed by extensive pathological examination and residual disease may be maximally reduced by surgical removal [13]. The patient and disease characteristics of those entered into this combined modality regimen are listed in Table 3.

Table 3. Disease characteristics prior to chemotherapy and response to HexaPAMP

	Remission evaluable ($n = 53$)	Abdominal radiation according to protocol ($n = 18$)
A. *Disease characteristics*		
Total hysterectomy + bilateral adnex + oophorectomy		
complete	32	11
incomplete	21	7
Residual tumor		
<2 cm	20	9
$\geqslant 2$ cm	33	9
Stage		
IIb+c	5	4
III	37	12
IV	11	2
Grade		
1	2	2
2+3	25	7
4	26	9
B. *Response to CT (II-look for responders)*		
CR	13	8
PRned	5	4
PR	13	4
NC	2	0
PD	19	1
Unknown (minimal residual at 1st operation)	1	1

Combination chemotherapy will select for resistant tumor subpopulations. Thus, switching modalities at or near the nadir achievable with a remission-inducing combination may limit the number of treatment failures and increase the probability of cure. Since residual disease and subsequent relapses are generally confined to the abdominal cavity [17], a whole-abdominal radiation therapy is administered with the intent to consolidate remission. As previously noted, only those patients with minimal residual disease can expect to benefit from this modality. Table 4 lists the radiation doses required to potentially eradicate residual abdominal disease. The tolerable doses of radiation for selected organs are listed in Table 5. Based upon this information, patients are selected for radiation after chemotherapy and second-look operation if:

Table 4. Relationship between tumor mass and potentially tumoricidal dose of radiation

	Diameter of tumor	Tumoricidal dose in Gy
Occult peritoneal metastases	$\leqslant 1$ mm	25
Occult nodal metastases	< 5 mm	$\geqslant 40$
Tumor, metastases	$\leqslant 1$ cm	50
Tumor, metastases	1–2 cm	60
Tumor, metastases	3 cm	$\geqslant 70$

The tumoricidal dosages are based upon the total dose given in 2.0–2.5 Gy/fraction by the moving strip technique.

Table 5. Whole-abdominal irradiation (moving strip technique, 22–24 Gy/10 sessions/12–14 days): tolerance and dose

Organ	Tolerance dose in Gy	Possible side effects of higher doses
Bone marrow[1]		Myelosuppression, aplasia
Liver	26–30	Hepatitis, fibrosis
Lung[2]	20–30	Pneumonitis, fibrosis
Heart	35–40	Pericarditis, pancarditis
Kidneys[3]	15–20	Acute and chronic nephritis
Stomach[4]	> 40	Ulceration
Small intestine[4]	30–40	Enteritis, stricture, perforation
Large intestine	45	Stricture, perforation
Bladder	> 50	Cystitis

[1] 50%–60% of the bone marrow lies within the radiated volume. Radiation is not given if leukocytes are $< 1500/mm^3$ and platelets $< 100{,}000/mm^3$.

[2] Only partially in irradiation field.

[3] Blocked by dorsal shielding.

[4] $> 80\%$ of patients with acute reactions (nausea, vomiting, diarrhea of some grade).

1) there has been a complete remission, or
2) there has been a partial remission and
   a) all detectable residual disease has been removed, or
   b) the remaining residual disease consists of tumor masses < 1 cm in diameter, or
   c) there was no response to chemotherapy but the residual disease was surgically reduced to masses < 1 cm in diameter.

Our radiation schedule consists of whole-abdominal irradiation with the 'moving strip' technique. Beginning 1 cm above the diaphragms without liver shielding the length of the irradiation field is extended from 4 cm to 10 cm, with *2 cm* strips (Table 6). A daily midplane dose of 2.2 Gy is given in each of 10 fractions in 12–14 days. Scattered radiation and beam divergence raise the total dose to 25–26 Gy. This schedule delivers tumoricidal yet tolerable doses to the upper abdomen which contains the peritoneal-surfaces of the diaphragms and the liver. The feasibility of this technique was initially piloted in patients who had no detectable or only minimal residual disease after primary surgery and in patients who had received various other

Table 6. Whole-abdominal irradiation of ovarian cancer moving strip technique, daily AP and PA, no liver shielding, PA renal shielding, daily dose 2.2 Gy (midplane)

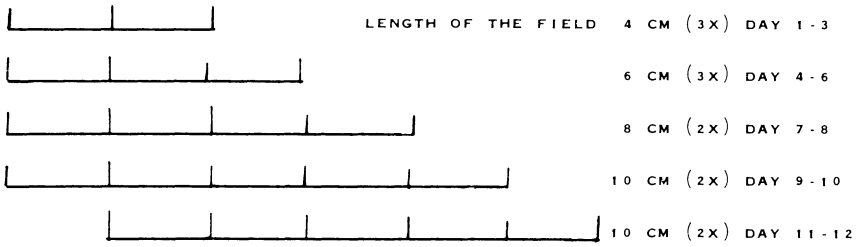


Table 7. Delivery of whole-abdominal radiation: incidence of treatment interruption

	Treatment prior to radiotherapy			
Radiotherapy	Laparotomy without CT's* (%)	Various CT's* (%)	HexaPAMP induction (%)	Total (%)
Delivered as planned	8 (53)	4 (40)	2 (11)	14 (32)
Temporary interruption	7 (47)	4 (40)	12 (67)	23 (54)
Not completed	0 (0)	2 (20)	4 (22)	6 (14)

* CT = chemotherapy, including L-PAM p.o.; HexaCAF; CHAD; VAC; adriamycin; DDP.

Table 8. Combined modality treatment of ovarian cancer.

Author (ref.) year stage	Treatment	No. patients		No. (%) of pts with minimal residual disease or no disease who completed all modalities	Results
		Included in trial	Irradiated +evaluable		
1) Tak [18] 1977 all stages	– ip+iv Thiotepa (short term) + MS+PB	60	60 (?)	30 (?) (50%)	3y survival rates 60% for minimal disease 8% for bulky disease
	– ACTD,C,F,V (short term) + AR (+boost)	60	60(?)	28 (?) (47%)	60% for minimal disease 8% for bulky disease
2) Brady [19] 1979 III	–AR (+boost) + L-PAM (until prog)	58	19	10 (53%)	median survival 14.0 mos. for min. disease 18.1 mos. for bulky disease
	– L-PAM (3 cycles) + AR (+boost)	62	15	4 (27%)	30.0 mos. for min. disease 15.5 mos. for bulky disease
3) Fuks [20] 1982 III	– CHAD (×6 cycles) + AR (open field)	15	7	7 (100%)	6/7 NED 14–27 mos. follow-up
4) Torres [21] 1982 IIIA	– ip 32p±ovarian antitumorserum (HOATS) + AR (split course) + L-PAM for 1 y	23	14	14 (100%)	6 who completed all modalities are NED and alive 15–59 mos after entry

ACTD = Actinomycin D; C = Cyclophosphamide; F = 5-Fluorouracil; V = Vindesine; H, Hexa = Hexamethylmelamine; A = Adriamycin; DDP = cis-platinum; L-PAM, PAM = Melphalan; AR = Abdominal radiation; MS = Moving strip; PB = pelvic boost; iv = intravenous; ip = intraperitoneal; NED = no evidence of disease.

Table 8. (continued)

Author (ref.) year stage	Treatment	No. patients: Included in trial	No. patients: Irradiated +evaluable	No. (%) of pts with minimal residual disease or no disease who completed all modalities	Results
5) Nevin [22] 1983	– L-PAM (iv) (×6 cycles) + MS				
I+II		13	9	9 (100%)	9 (+4 without RT) NED at mean 5y (range 2–10 y)
III		37	21	18 (86%)	4/17PRs* and 10/10 CRs* at mean 5y (range 2–10y) (* all 14 survivors had minimal disease after L-PAM)
6) Hainsworth [23] 1983 III	– HCAP or HFAP (6 cycles) 'open field' AR	17	17	6 (35%) no or microscopic disease; 11 (65%) nodules <2 cm	3/17 are NED 9,14,21 mos after radiotherapy
7) Vogl [24] 1983 III	– AR 'split course' with (between courses) *or* after CHD or CHAD	25 (?)	25	11 (44%) no 'gross' disease	5 are NED med. 28+mos. (13–37 mos) after radiotherapy, 3 developed leukemia
8) Greiner [25] 1983 IIb+III+IV	– HexaPAMP	53	18	15 (83%) no or minimal disease 3 (17%) unknown residual tumors	12/18 are NED, med. 24+mos (range 16–37+mos.) after chemotherapy

ACTD = Actinomycin D; C = Cyclophosphamide; F = 5-Fluorouracil; V = Vindesine; H, Hexa = Hexamethylmelamine; A = Adriamycin; P, DDP = cis-platinum; L-PAM, PAM = Melphalan; AR = Abdominal radiation; MS = Moving strip; PB = pelvic boost; iv = intravenous; ip = intraperitoneal; NED = no evidence of disease.

chemotherapies (Table 7). This demonstrated that a majority (79%) of the patients pretreated with chemotherapy will finish the radiation therapy. In the interest of shortening the duration of radiation therapy , we are piloting application of the 'open field' whole-abdominal radiation technique [9].

Results of combined modality approaches

The published results of the studies using a multimodal approach are listed in Table 8. These results have been focused on those treated patients with no or with only minimal residual disease prior to radiation therapy. In general, the combined modality approach appears to be effective in these patients, but the majority of studies have only short-term results. The only long-term results available are those of Nevin *et al.* [22]. In this study, 38% (14/37) of the Stage III patients are 'long-term' survivors for 3 or more years. Furthermore, these patients are 66% (14/21) of the patients who received both chemotherapy and radiation therapy. In striking contrast to these results, Hainsworth *et al.* [23] found that only 18% (3/17) of their patients with 'advanced minimal residual disease' after second-look laparotomy are alive with short-term follow-up after radiation therapy. In our study, 18 patients have completed the ongoing combined modality protocol. As seen in Table 3, 13 patients had no detectable disease prior to the initiation of radiation therapy. Twelve of these patients are alive NED at a median of 24 months (range 16–37 mos) from study entry. One patient developed symptoms of a subsequently proven recurrence at 24 months follow-up. In this patient the planned radiation was not completed due to thrombocytopenia. Three of the six patients with residual disease after second-look laparotomy have relapsed within 28 months from study entry. Two of these had diffuse residual disease and one patient had residual disease of unknown amount.

Table 9a. Hematological toxicity observed during HexaPAMP chemotherapy

	Median	Range	Comments
Hb drop	3.3 g%	(1.0–8.4)	
WBC	$1.9 \times 10^3/mm^3$	$(0.2–3.2 \times 10^3)$	Median nadir on Day 80 10 pts had $<1000/mm^3$
Platelets	$51 \times 10^3/mm^3$	$(3.5–245 \times 10^3)$	Median nadir on Day 92 12 pts had $<25000/mm^3$ 39 pts had $<100000/mm^3$

Toxicity

Dose and schedule are critical factors in cancer therapy. The dose-response curve for almost all known treatment modalities is steep for both the toxicity and therapeutic effects. In fact multiple drug combinations in remission induction schedules produce more severe myelotoxicity and nausea and vomiting than single-agent chemotherapy [22–24]. The toxicity encountered during the remission induction schedule of our combined modality protocol are presented in Table 9. Noteworthy is a severe, irreversible peripheral neurotoxicity which occurred in 5/51 patients in the form of paresthesias and ataxia. Sural nerve biopsy in one of these patients revealed axonal degeneration.

Various authors indicate that an increased incidence of severe toxicity may be observed during whole-abdominal irradiation in those patients who have received prior chemotherapy. Thrombocytopenia during radiation therapy is the most common treatment limiting toxicity, whereas gastrointestinal symptoms, especially diarrhea and abdominal pain, only rarely are treatment-limiting [19, 20, 23]. In our patients who had received any prior

Table 9b. Non-hematological toxicity observed during and after HexaPAMP chemotherapy

Toxicity	No. pts (%)
Nausea/vomiting (moderate–severe)	All
Nephrotoxicity	
— reversible	9 (18)
— irreversible	2 (2)
— salt wasting syndrome (hypomagnesemia)	2 (4)
Neurotoxicity	
— paresthesia/ataxia irreversible	5 (10)
— paresthesia-reversible	4 (8)
— hypoacusia/tinnitus	3 (6)
— confusion	1 (2)
Alopecia	
— complete	14 (27)
— incomplete	13 (25)
Others	
— cardiac arrest	1 (2)
— myocardial infarction	1 (2)
— iridocyclitis/glaucoma	1 (2)
— cerebral hematoma	1 (2)
Toxic death	
— died at home (toxic death cannot be excluded)	1 (2)

chemotherapy, the most significant toxicity observed during radiation therapy was hematological (Table 10). Thrombocytopenia was the primary cause of at least a temporary interruption in the planned radiation therapy in 18/28 (64%) patients who received prior chemotherapy and 7/15 (47%) in the group receiving radiation therapy alone (Table 11). In the chemotherapy-treated patients who did not finish the radiotherapy, interruptions were due to thrombocytopenia in 4 patients and abdominal symptoms in 2 patients (Table 7). Our experience suggests that prolonged thrombocytopenia may result if radiation therapy is continued after the platelet count falls below 100,000/mm^3. As a result radiation therapy may not be completed (Table 11).

Significant late-appearing complications from a combined modality ap-

Table 10. Toxicity observed during and after radiation therapy

Findings and symptoms	Treatment prior to radiotherapy		
	No prior chemotherapy ($n = 15$) (%)	Prior HexaPAMP* ($n = 18$) (%)	Prior various chemotherapies ** ($n = 10$) (%)
Thrombocytopenia			
$<50{,}000/mm^3$	3 (20)	4 (22)	6 (60)
$<20{,}000/mm^3$	1 (7)	4 (22)	5 (50)
Leucopenia			
$<2{,}000/mm^3$	7 (47)	10 (56)	6 (60)
Loss of <5% body weight	4 (27)	4 (22)	1 (10)
Heavy tiredness	4 (27)	6 (33)	6 (60)
Intestinal symptoms			
(≥moderate)	3 (20)	4 (22)	4 (40)
Chronic ileus	1 (7)	3 (17)	1 (10)
Alkal. phosphatase			
>1.5-fold increase	5 (33)	7 (33)	5 (50)
Creatinine increase			
>100 µmol/l	1 (7)	3 (17)	1 (10)
>120 µmol/l	– –	1† (6)	– –
Basal pneumonitis	8 (53)	4 (22)	4 (40)
Benign ascites	2 (13)	1 (6)	1 (10)
Pericarditis	1 (7)	– –	– –

* Hexamthylmelamine, L-PAM, and DDP.
** Included L-PAM; HexaCAF; CHAD.
† Previous chronic pyelonephritis.

Table 11. Thrombocytopenia as limiting toxicity of abdominal radiation

	Platelet count limit at interruption of radiation therapy:	
	80–100,000/m^3	> 50,000/mm^3
No. of patients	18	7
Subsequent platelet nadir		
range (/mm^3)	$2–83 \times 10^3$	$5–22 \times 10^3$
median	58×10^3	10×10^3
Treatment delay (days)		
range	13–50	31–115
median	29	66
Treatment terminated	1/18	3/7

proach may include bowel obstruction and leukemia. In the series of Tak *et al.* [18], 6% (8/120) of the patients required surgery for severe abdominal complications (5 died after bowel resections and 3 survived the complication after bypass operations). In our series 2 patients required abdominal surgery for complications of the combined therapy (intestinal adhesions). One recent series by Vogl *et al.* [24] reported leukemia in three patients who received an alternating course of radiation and combination chemotherapy.

A remission induction schedule that maximizes the reduction of detectable tumor mass prior to radiation therapy will be toxic. Thus, it is important to identify those patients who will not benefit from such a schedule. Specific histological types of ovarian epithelial cancers have preliminarily been identified as nonresponders to the HexaPAMP chemotherapy. Seven of seven patients with clear cell carcinomas and three of three patients with malignant mixed Mullerian tumors have experienced progressive disease during this remission induction schedule [26].

Perspective

From consideration of the important aspects of the therapeutic approach against advanced ovarian carcinoma, the following have emerged:

1) a significant remission is attainable with a combination chemotherapy,
2) complete remission does not necessarily mean cure,
3) radiation therapy is effective in eradication of microscopic residual disease, and may therefore consolidate remission.

References

1. Neijt JP, Ten Bokkel Huinink WM, van der Burg MEL, van Oosterom AT, Vriesendorp R, Boven E, Kooyman CD, van Honurelingen JC, Pinedo HM: Combination chemotherapy with hexa-CAF and CHAP-5 in advanced ovarian carcinoma: A randomized study of the Netherlands joint study group for ovarian cancer. Proc ASCO 2:148, 1983.
2. Barker GH, Wiltshaw E: Randomized trial comparing low-dose cis-platin and chlorambucil with low-dose cis-platin, chlorambucil and doxorubicin in advanced ovarian carcinoma. Lancet i:747–750, 1981.
3. Williams CJ, Mead B, Arnold A, Green J, Buchanan R, Whitehouse M: Chemotherapy of advanced ovarian carcinoma: initial experience using a platinum-based combination. Cancer 49:1778–1783, 1982.
4. Young RC, Chabner BA, Hubbard SP, Fisher RI, Bender RA, Anderson T, Simon RM, Canellos GP, DeVita VT: Advanced ovarian adenocarcinoma; A prospective clinical trial of melphalan (L-PAM) versus combination chemotherapy. New Engl J Med 293:1261–1266, 1978.
5. Stehman FB, Ehrlich CE, Einhorn LH, Williams SD, Roth LM: Long-term follow-up and survival in Stage III-IV epithelial ovarian cancer treated with cis-dichlorodiammine platinum, adriamycin and cyclosphamide (PAC). Proc ASCO 2:147, 1983.
6. Greco A: Personal communication.
7. Van der Schueren E, Van cer Bogaert W, Gonzales D: Role of radiotherapy in the treatment of ovarian carcinoma. In: Ovarian Cancer; Advances in the Biosciences, Vol 26. Edited by CE Newman, CHJ Ford, JA Jordan. Pergamon Press, New York 1980, pp 151–164.
8. Dembo AJ, Bush RS, Beale FA, Bean HA, Pringle JF, Sturgeon JFG: The Princess Margaret Hospital Study of ovarian Cancer: Stage I, II and Asymptomatic III presentations. Cancer Treat Rep 63:249–254, 1979.
9. Dembo AJ, Bush RS, Beale FA, Bean HA, Fine S, Gospodarowicz M, Herman J, Pringle JF, Sturgeon J, Thomas GM: A ramdomized clinical trial of moving-strip versus open field whole-abdominal irradiation in patients with invasive epithelial cancer of ovary. Proc ASCO 2:146, 1983.
10. Dembo A: Personal communication.
11. Skipper HE: Southern Research Institute, Booklet 4 (1980).
12. Griffiths CT, Fuller AF: Intensive surgical and chemotherapeutic management of advanced ovarian cancer. Surg Clin North Am 58:131–142, 1978.
13. Smith JP: Surgery for ovarian cancer. In: Newman CE, Ford CHJ, Jordan JA (eds) Ovarian Cancer, Advances in the Biosciences, Vol 26: Pergamon Press, New York, 1980, pp 137–149.
14. Goldie JH, Coldman AJ: A mathematic model for relating the drug sensitivity of tumors to their spontaneous mutation rate. Cancer Treat Rep 63:1727–1733, 1979.
15. Tattersall MHN, Jarman M, Newlands ES: Pharmaco-kinetics of melphalan following oral or intravenous administtration in patients with malignant disease. Eur J Cancer 14:507–513, 1978.
16. Alberts DS, Chen HSG, Benz D, Mason NL: Effect of renal dysfunction in dogs on the disposition and marrow toxicity of melphalan. Br J Cancer 43:330–334, 1981.
17. Fuks Z: Patterns of spread of ovarian carcinoma: relation to therapeutic strategies. In: Newman CE, Ford CHJ, Jordan JA (eds) Ovarian Cancer. Pergamon Press, Oxford, New York, 1980, pp 39–56.
18. Tak WK, Costanza ME, Marchant DJ, Munzenrider JE, Mitchell GW, Nathanson L, Emami B: Surgery, chemotherapy and supervoltage radiotherapy for carcinoma of the ovary. Int J Radiation Oncology Biol Phys 2:895–901, 1977.
19. Brady LW, Homesley HD, Blessing JA, Lewis GC, Slayton RE, for the GOG: Radiotherapy (RT), chemotherapy (CT), and combined therapy in Stage III epithelial ovarian cancer.

Cancer Clin Trials 1:111–120, 1979.
20. Fuks Z, Rizel S, Anteby SO, Biran S: The multimodal approach to the treatment of Stage III ovarian carcinoma. Int J Radiation Oncol Biol Phys 8:903–908, 1982.
21. Pino y Torres JL, Bross DS, Hernandez E, Rosendhein N, Bross DS, Klein JL, Ettinger DS, Leichner PK, Order SE: Multimodality treatment of patients with advanced ovarian carcinoma. Int J Radiation Oncol Biol Phys 8:1671–1677, 1982.
22. Nevin JE, Pinzon G, Baggerly TJ, Turner FG, Marsella JJ: The use of intravenous phenylalanine mustard followed by supervoltage irradiation in the treatment of carcinoma of the ovary. Cancer 51:1273–1283, 1983.
23. Hainsworth JB, Malcolm A, Johnson DH, Burnett LS, Jones HW, Greco FA: Advanced minimal residual ovarian carcinoma: abdominopelvic irradiation following combination chemotherapy. Obst Gynecol 61:619–621, 1983.
24. Vogl SE, Seltzer V, Greenwald E, Kaplan B, Abitbol A: Consolidation radiatherapy and chemotherapy (CT) for ovarian cancer after CT-induced remission: long-term remission of initial bulky disease. Proc ASCO 2:151, 1983.
25. Greiner R, Goldhirsch A, Dreher E, Davis B, Locher G, Payer T, Neuenschwander H, Joss R, Brunner K, Veraguth P: Whole abdominal radiation in patients with advanced ovarian carcinoma after surgery, chemotherapy and second-look laparotomy. J Cancer Res Clin Oncol 107:94–98, 1984.
26. Davis B, Goldhirsch A, Locher G, Dreher E, Greiner R, Brunner K: Prognostic factors for remission induction in advanced ovarian cancer. J Cancer Res Clin Oncol 107:106–110, 1984.

Addendum

As of september 1984 76 patients are fully evaluable for response. The pathologically proven complete response rate exceeds 30% and none of the complete remitters who completed radiation therapy have relapsed.

11. Malignant germ cell tumors of the ovary

DAVID M. GERSHENSON and J. TAYLOR WHARTON

1. Introduction

Malignant germ cell tumors of the ovary are those neoplasms derived from the primitive germ cells of the embryonic gonad. As a group they represent less than 5% of all ovarian malignancies [1]. Younger patients with ovarian tumors, however, have a much greater likelihood of having a malignant germ cell tumor [2, 3].

These tumors generally occur in girls and young women and are considered to be highly malignant and rapidly growing. Because of the rarity of these tumors, only recently have we been able to appreciate their biologic behavior and to clarify their terminology. Over the last 3 decades these aspects have been refined and modern treatment has evolved.

The concept of germ cell tumors as a specific group of ovarian neoplasms is based, as suggested by Teilum [4], on their common histogenesis (Figure 1), on the presence of histologically different elements within the same neoplasm, on the occurrence of histologically similar neoplasms in extra-gonadal sites along the line of migration of the primitive germ cells [5, 6], and on homology between specific tumor types in the different sexes [7–10].

The currently accepted classification of germ cell tumors of the ovary was

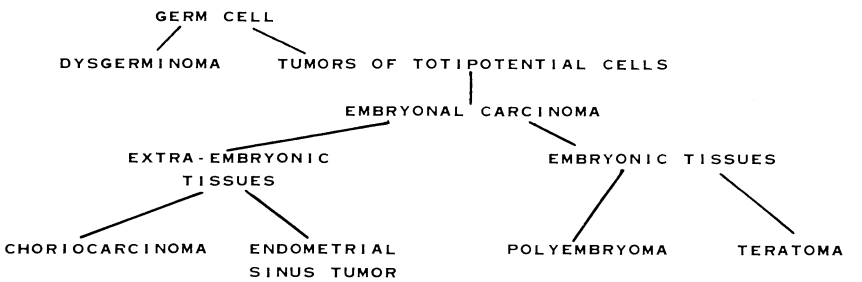


Figure 1. Histogenesis of germ cell tumors of the ovary. Modified from G. Teilum: Acta Pathol Microbiol Scand 65:497, 1965.

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introduced by the World Health Organization in 1973 [11]. This was a major advance in terms of standardization of nomenclature and histologic criteria. The present chapter will present clinicopathologic features of each of the malignant germ cell tumors based on the experience at The University of Texas M.D. Anderson Hospital and Tumor Institute from 1944 to 1983 with 216 patients with these rare neoplasms (Table 1).

2. Dysgerminoma

Dysgerminoma, the most common malignant germ cell tumor, accounted for 34% of all germ cell tumor cases seen at UT M.D. Anderson Hospital. In 1911 Chenot [12] first described this neoplasm. Meyer [13] then introduced the term dysgerminoma in 1931. It accounts for approximately 2% of all ovarian malignancies and is the homologue of the male seminoma. It seems to be unique among malignant ovarian germ cell tumors for its incidence of bilaterality and its radiosensitivity.

Dysgerminoma is composed of cells resembling primordial germ cells which contain large amounts of glycogen. Among malignant germ cell tumors, dysgerminoma is most commonly associated with dysgenetic gonads and a Y-chromosome.

2.1. Clinical profile

There were 74 patients in the UT M.D. Anderson Hospital series with pure dysgerminoma. The age of the patients ranged from 7 to 46 years with a median of 17 years. Forty-six patients were white, 22 Hispanic, 5 black and 1 Indian. It is one of the most common ovarian tumors diagnosed in pregnancy and was associated with pregnancy in 9 patients in this series – 3

Table 1. Malignant germ cell tumors – UT M.D. Anderson Hospital Series 1944–1983

Histology	No. patients
Dysgerminoma	74
Immature teratoma	51
Endodermal sinus tumor	45
Mixed germ cell tumor	42
Choriocarcinoma	2
Embryonal carcinoma	1
Polyembryoma	1
Total	216

during the later stages of pregnancy, 5 at the time of delivery, and 1 in the postpartum period.

Abdominal pain was present in 63 (85%) patients. In 3 of these patients the pain presented as an acute abdomen. A palpable mass was noted in 59 (80%) patients, 12 of whom were asymptomatic. Abdominal distension occurred in 22 (30%) patients. Fever and vaginal bleeding occurred in 3 (4%) patients each. The duration of symptoms ranged from 1 day to 6 months, with a median duration of 4 weeks.

2.2. Operative findings

The tumor arose in the right ovary in 38 patients and in the left ovary in 25 patients. In 9 patients (12%) bilateral ovarian involvement was noted. In 2 cases the side of involvement was unknown. In 7 cases (9%) the tumor ruptured – 5 preoperatively and 2 intraoperatively. Ascites was present in 8 patients and torsion of the ovarian pedicle was observed in 2 patients. Forty-six of the neoplasms (62%) were stage I, 5 tumors (7%) were stage II, 18 tumors (24%) were stage III, and 4 tumors (5%) were stage IV. Stage was undetermined in 1 patient.

2.3. Pathology

The greatest diameter of the tumors ranged from 7 to 36 cm, with a median of 15 cm. Macroscopically, dysgerminoma is usually unilateral, although it may be bilateral in 10%–15% cases. Its external surface is generally smooth and lobulated with a gray-white capsule. On cut surface it is solid, pink or tan, and has focal hemorrhage or necrosis. Calcifications suggest the presence of an underlying gonadoblastoma. In this series underlying gonadoblastoma was documented in 2 cases and associated with 46XY karyotype. Microscopically, the appearance is quite distinct and identical to the testicular seminoma (Figure 2). It is composed of aggregates or islands of large round or polygonal cells with abundant glycogen and a prominent cell membrane surrounded by fibrous septae containing lymphocytes. Occasionally, syncytiotrophoblastic giant cells which may stain for immunoperoxidase are present. These areas, whenever present, are responsible for hCG production. Areas lacking cytotrophoblast should not be confused with choriocarcinoma. As noted, areas of hemorrhage, necrosis, and occasional calcification may be seen. Dysgerminoma may occasionally be confused with lymphoma, an error that should be carefully avoided, since treatment differences may severely compromise survival probability.

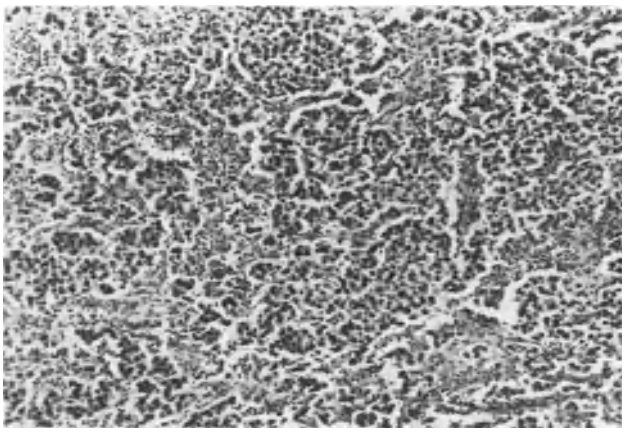

Figure 2. Dysgerminoma. Irregular clusters of primitive germ cells are separated by thin fibrous septae containing lymphocytes (H& E, × 50).

3. Endodermal sinus tumor

Pure endodermal sinus tumor accounted for 21% of all germ cell tumor cases seen at UT M.D. Anderson Hospital, representing the third most common tumor in this series. In the AFIP series it ranked second in frequency to dysgerminoma [14, 15].

In 1939, Schiller [16] described a group of ovarian tumors for which he proposed a mesonephric origin. In 1946, Teilum [8] indicated that the tumor described as mesonephroma included the more common clear cell carcinoma as well as germ cell tumors. He refuted the mesonephric origin and described the extra-embryonic germ cell origin of this tumor, which he noted resembled the endodermal sinuses of Duval in the yolk sac of the rat placenta [4, 17, 18]. The association of elevated serum levels of alpha-fetoprotein with its immunohistochemical identification in tumor cells has strengthened the evidence suggesting the close relationship to the yolk sac [14, 19–24].

The study of the histogenesis and biologic behavior of endodermal sinus tumor and the development of treatment principles have been hindered by the confusion regarding terminology and the multiplicity of histologic patterns. Only with the recent reports of several large series [14, 25, 26] added to scattered case reports have we begun to fully appreciate the natural history of this disease. Although older reports [27, 28] indiscriminantly included endodermal sinus tumors with embryonal carcinomas or mixed tumors, Kurman and Norris [29] clearly distinguished embryonal carcinoma from endodermal sinus tumor.

3.1. Clinical profile

There were 45 patients in the UT M.D. Anderson Hospital series with pure endodermal sinus tumor. The age of the patients ranged from 7 to 37 years with a median of 19 years. Thirty patients were white, 6 black, 8 Hispanic, and 1 Oriental. Association of the tumor with pregnancy occurred in 3 patients.

Abdominal pain was the most common symptom, occurring in 37 (82%) patients. In 5 of these patients the pain presented as an acute abdomen. A palpable mass was noted in 34 (76%) patients, 4 of whom were asymptomatic. Abdominal distention was present in 17 (38%) patients. Fever and vaginal bleeding occurred in 14 (31%) and 2 (4%), respectively. The duration of symptoms ranged from 2 days to 6 months, with a median duration of 4 weeks.

3.2. Operative findings

The tumor arose in the right ovary in 19 patients and in the left ovary in 25 patients. In 1 patient information concerning site of origin was not available. No case of bilateral ovarian involvement was noted. In 16 cases (36%) the tumor ruptured – 4 preoperatively and 12 intraoperatively. Torsion of the ovarian pedicle was documented in 2 cases. Ascites was present in 12 patients.

Twenty-five of the neoplasms (56%) were stage I, 5 tumors (11%) were stage II, and 15 tumors (33%) were stage III. There were no stage IV neoplasms in this series.

3.3. Pathology

The greatest diameter of the tumor ranged from 9 to 40 cm, with a median of 16 cm. These neoplasms are usually encapsulated, smooth, and lobulated. The cut surface is usually tan to gray-yellow with a variegated appearance, with cystic and solid areas and extensive areas of hemorrhage and necrosis. Benign cystic teratoma is not infrequently associated with endodermal sinus tumor. In this series there were 6 such cases – 2 in the ipsilateral ovary, 2 in the contralateral ovary, and 2 in the ovaries bilaterally. Moreover, 2 additional patients developed dermoids in the contralateral residual ovary several years following initial treatment for endodermal sinus tumor.

Microscopically there are five principal patterns, as described by Teilum [17, 18]. The most common is the reticular pattern, characterized by a

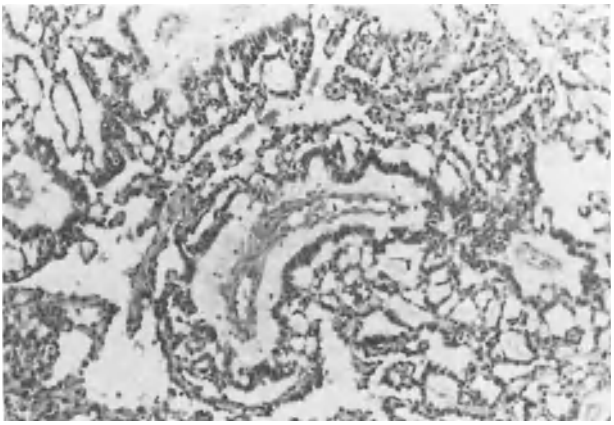

Figure 3. Endodermal Sinus Tumor. Loose reticular pattern with a centrally-placed longitudinally-sectioned Schiller Duval body (H & , × 50).

meshwork of empty vacuoles lined by flattened cells (Figure 3). The festoon or endodermal sinus pattern consists of undulating epithelial cells often associated with Schiller-Duval bodies. The polyvesicular vitelline pattern is rare, composed of numerous cysts or vesicles surrounded by dense fibroblastic stroma. The alveolar-glandular type consists of cystic spaces and cavities lined by flat or cuboidal cells and surrounded by myxomatous stroma. The solid pattern is composed of undifferentiated embryonal cells. These patterns may exist in the pure state, but more commonly multiple patterns are observed in the same tumor, with one or two predominating. There is no known difference in the various patterns with regard to prognosis. Hyaline bodies are very common. Immunochemical studies have clearly demonstrated that these droplets contain alpha-fetoprotein [14, 30]. Necrosis and hemorrhage are usually extensive.

4. Immature teratoma

Immature teratoma accounted for 24% of all germ cell tumor cases seen at UT M.D. Anderson Hospital, representing the second most common tumor in this series. The term denotes a pure teratoma that contains a variable amount of immature tissue derived from the three germ cell layers – ectoderm, mesoderm, and endoderm. These tumors represent approximately 1% of all ovarian teratomata. Like endodermal sinus tumor, the study of the behavior, treatment, and prognosis of this tumor has been hindered by confusion in terminology and histologic description. Terms commonly applied to this tumor include malignant teratoma (confusing it with malignant degeneration in a benign teratoma), solid teratoma (these

tumors are not uncommonly partially cystic), embryonal teratoma (potentially confusing it with embryonal carcinoma or endodermal sinus tumor), and teratocarcinoma (sometimes applied to mixed tumors). Many of the so-called immature teratomas described in the literature have undoubtedly included other types of germ cell tumors.

4.1. Clinical profile

There were 51 patients in the UT M.D. Anderson Hospital series with pure immature teratoma. The age of the patients ranged from 6 to 69 years with a median of 20 years. Forty-one patients were white, 7 Hispanic, and 3 black. Association of the tumor with pregnancy occurred in 1 patient.

Abdominal pain was present in 45 (88%) patients. In 2 of these patients the pain presented as an acute abdomen. A palpable mass was noted in 48 (94%) patients, 5 of whom were asymptomatic. Abdominal distension occurred in 26 (51%) patients. Fever and vaginal bleeding occurred in 3 (6%) and 6 (12%) patients, respectively. The duration of symptoms ranged from 2 days to 6 months, with a median duration of 4 weeks.

4.2. Operative findings

The tumor arose in the right ovary in 24 patients and in the left ovary in 20 patients. Seven patients had bilateral ovarian involvement. In 6 cases (12%) the tumor ruptured – 2 preoperatively and 4 intraoperatively. Torsion of the ovarian pedicle could be documented in 2 cases. Ascites was present in 10 patients. Twenty-seven of the neoplasms (53%) were stage I, 5 tumors (10%) were stage II, 18 tumors (35%) were stage III, and 1 tumor (2%) was stage IV.

4.3. Pathology

The greatest diameter of the tumors ranged from 8 to 40 cm, with a median of 16 cm. Macroscopically, these tumors are usually unilateral and lobulated with a smooth external surface. On cut surface they appear soft and fleshy, and varying in color from gray to dark brown. Hemorrhage and necrosis are common. They are principally solid, but with cystic areas of variable size. Bone or cartilage may be grossly recognizable.

As with other germ cell tumors, immature teratomas may be associated with a benign cystic teratoma. In the present series, this occurred in 6 cases (12%) – 1 in the ipsilateral ovary, 4 in the contralateral ovary, and 1 in the

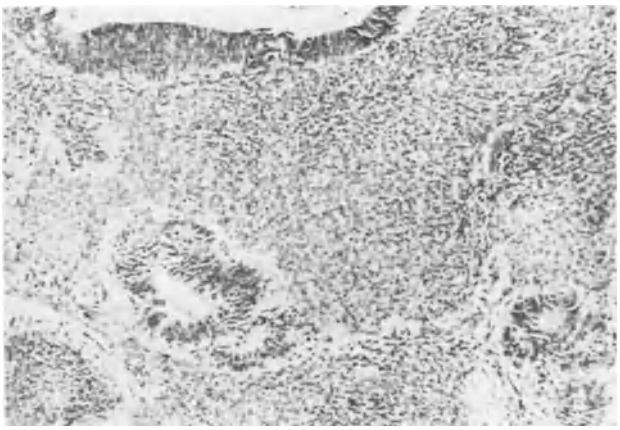

Figure 4. Immature Teratoma, grade 3. Several neuroepithelial rosettes are surrounded by cellular glial tissue (H & E, × 50).

ovaries bilaterally. Associated gonadoblastoma was present in 1 patient.

Microscopically, a wide range of tissues representing the 3 germ cell layers with varying degrees of differentiation may be identified (Figure 4). Neuroepithelium or immature mesenchymal tissue may predominate. Immature epithelium, cartilage, or muscle may also be encountered.

A grading system relating the degree of immaturity of the teratoma to its biologic behavior was initially proposed by Thurlbeck and Scully [31], later amplified by Robboy and Scully [32], and subsequently modified by Norris *et al.* [33] (Table 2). The latter authors have suggested that the histologic grade of the primary lesion determines the likelihood of extraovarian spread, and when extraovarian spread has occurred, the predominant prognostic factor is the grade of the metastatic disease.

Adequate grading of immature teratoma depends upon adequate tissue sampling, taking one section for every 1 cm of maximum tumor diameter.

Table 2. Grading system for immature teratoma

Grade 0:	All tissues mature; no mitotic activity.
Grade 1:	Abundant mature tissue but with some immaturity, mainly glial, with loose, primitive mesenchyme. Mitoses present, but neuroepithelium is absent or restricted to one low power field (40×) per slide.
Grade 2:	Greater immaturity, with neuroepithelium not exceeding 3 low power fields per slide.
Grade 3:	Severe immaturity, with neuroepithelium found in 4 or more low power fields per slide and frequently merging with sarcomatous stroma.

Adapted from Norris et al. 1976 [33].

Moreover, such meticulous sampling and analysis will detect any other germ cell elements present.

5. Mixed germ cell tumor

Mixed germ cell tumors accounted for 19% of all germ cell tumor cases seen at UT M.D. Anderson Hospital, representing the fourth most common tumor in this series. In the AFIP series it accounted for only 8% of malignant germ cell tumors [34].

These tumors contain 2 or more malignant germ cell components. The combination of a benign germ cell component or gonadoblastoma with only one malignant element does not qualify as a mixed germ cell tumor. The various elements present in these tumors may be intimately admixed or may occur in adjacent but separate areas of the neoplasm. Although, in the past, several mixed tumors were classified according to the predominant element alone, thorough sampling is critical. Inadequate sampling may lead, for instance, to the erroneous diagnosis of pure dysgerminoma for which overall treatment and prognosis might be quite different. Therefore, areas which grossly appear dissimilar should be carefully sampled and thoroughly analyzed. The number, type, and estimated percent composition of each element should be stated. Only in this manner will we continue to gain knowledge concerning the behavior, treatment, and prognosis associated with various elements. Although, in the past, mixed tumors were thought to be uncommon, more extensive examination of germ cell tumors will undoubtedly result in higher frequencies within most large series.

5. Clinical profile

There were 42 patients in the UT M.D. Anderson Hospital series with malignant mixed germ cell tumors. The age of the patients ranged from 6 to 31 years with a median of 16 years. Twenty-seven patients were white, 11 Hispanic, and 4 black. Association of the tumor with pregnancy occurred in 2 patients.

Abdominal pain was the most common symptom, occurring in 38 (90%) patients. In 6 of these patients the pain presented as an acute abdomen. A palpable mass was noted in 37 (88%) patients, 1 of whom was asymptomatic. Abdominal distension was present in 11 (26%) patients. Fever, vaginal bleeding, and amenorrhea occurred in 5 (12%), 6 (14%), and 3 (4%) patients, respectively. Two (5%) patients exhibited precocious puberty. The duration of symptoms ranged from 1 day to 6 months, with a median duration of 4 weeks.

5.2. Operative findings

The tumor arose in the right ovary in 21 patients, and in the left ovary in 13 patients. In 8 patients bilateral ovarian involvement was noted. In 12 cases (29%) the tumor ruptured – 6 preoperatively and 6 intraoperatively. Torsion of the ovarian pedicle could be documented in 3 cases. Ascites was present in 9 patients.

Twenty-four of the neoplasms (57%) were stage I, 4 tumors (10%) were stage II, 10 tumors (24%) were stage III, and 4 tumors (10%) were stage IV.

5.3. Pathology

The greatest diameter of the tumors ranged from 7 to 35 cm, with a median of 15 cm. The external surface is usually smooth and encapsulated. The cut surface is quite variable, depending on the type and quantity of the components. Dysgerminoma is usually soft, solid, and tan. Endodermal sinus tumor is hemorrhagic and necrotic, and sometimes gelatinous. Immature teratoma is almost always partially cystic. Choriocarcinoma and embryonal carcinoma are also necrotic and hemorrhagic. Benign cystic teratoma may be present in association with mixed tumors. In the present series, this occurred in 3 cases – 1 in the ipsilateral ovary and 2 in the contralateral ovary. Associated gonadoblastoma was present in 3 patients with 46XY karyotype.

Histologically, by definition, at least 2 malignant elements are present. In this series, 2 malignant components were present in 81% of the tumors, 3 components were present in 14% of the tumors, and 4 or 5 components were each present in 2% of the tumors. Dysgerminoma was found in 69% of the tumors, followed by immature teratoma in 62%, endodermal sinus tumor in 60%, embryonal carcinoma in 24%, and choriocarcinoma in 10%. Figure 5 represents a typical histologic finding in this tumor.

6. Embryonal carcinoma

Although recognized in the 1973 World Health Organization Classification [11], only in the last few years has embryonal carcinoma been characterized as a separate clinicopathologic entity [29]. Previously the terms 'embryonal carcinoma' and 'endodermal sinus tumor' were used interchangeably. It is now evident that embryonal carcinoma of the ovary resembles the tumor of the same name occurring in the adult testis [35].

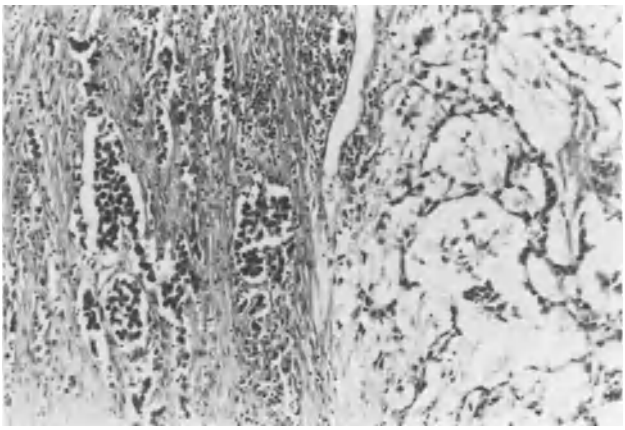

Figure 5. Malignant Mixed Germ Cell Tumor. Dysgerminoma and Endodermal Sinus Tumor. The dysgerminoma component is composed of small nests of hyperchromatic germ cells surrounded by fibrous stroma and scattered lymphycytes. The adjacent endodermal sinus tumor is of the loose reticular type (H & E, × 50).

Embryonal carcinoma is considered to be composed of totipotential cells capable of differentiation into embryonic tissues (i.e. teratoma or polyembryoma) or into extraembryonic tissues (i.e. endodermal sinus tumor or choriocarcinoma). Although it is a relatively rare component of mixed germ cell tumors, it is exceedingly rare in the pure state, as evidenced by the fact that only one pure embryonal carcinoma has been identified in the UT M.D. Anderson Hospital series. This patient was a white female, 15 years old at the time of diagnosis. She had a 4-month history of lower abdominal pain and irregular menses and was found to have a palpable mass on examination. Exploratory laparotomy revealed a 16 cm solid left ovarian tumor as well as metastatic implants on the posterior uterus, pelvic peritoneum, and cecum (stage III). Serum hCG level 1 week postoperatively was 3000 IU per liter.

In 1976 Kurman and Norris [29] reported the Armed Forces Institute of Pathology series of 15 embryonal carcinomas, 11 of which were pure. Clinical and operative findings were similar to those of other patients with malignant germ cell tumors of the ovary. As with polyembryoma and nongestational choriocarcinoma, because of the production of chorionic gonadotropin, it is not uncommon for patients with these tumors to exhibit precocious puberty.

Grossly, the tumor may have a smooth external surface and may appear solid on sectioning with gray-white to yellow color and a granular texture. Areas of necrosis and hemorrhage are quite common. Cystic spaces may also occur. Histologically, this tumor is composed of solid sheets of large, primitive pleomorphic cells with granulated vacuolated cytoplasm (Figure

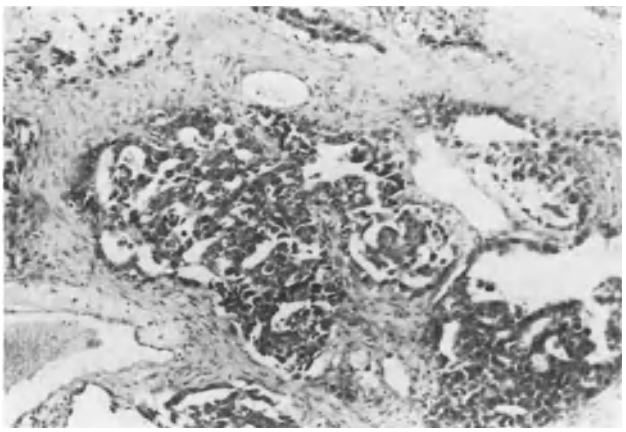

Figure 6. Embryonal Carcinoma. Undifferentiated cells with an adenocarcinomatous appearance (H & E, × 50).

6). The cells may form papillary processes and gland-like clefts. Nuclei are round, vesicular, and coarse. Other common features are the presence of multinucleated giant cells, hyaline bodies, and isolated clusters of syncytiotrophoblastic cells. Necrosis and hemorrhage are common. Immunoperoxidase studies demonstrate the presence of chorionic gonadotropin in syncytiotrophoblastic cells and less commonly the presence of alpha-fetoprotein in hyaline bodies.

7. Nongestational choriocarcinoma

In 1940 Pick [36] first described the presence of choriocarcinoma in an ovarain teratoma. Since that time scattered reports of ovarian choriocarcinoma have clarified the fact that it, too, is an exceedingly rare germ cell tumor, more commonly seen as a component of mixed tumors than in the pure state. It may originate as a gestational tumor associated with an ovarian pregnancy or having metastasized from a uterine or tubal primary lesion, or as a nongestational tumor, arising primarily in the ovary and not associated with a pregnancy.

If other germ cell elements are present, nongestational choriocarcinoma may be diagnosed confidently in patients of any age. The pure nongestational state, on the other hand, may only be diagnosed with certainty in the prepubertal patient.

Fox and Langley [37] have carefully studied the world literature and have found 40 acceptable examples of nongestational choriocarcinoma reported in some detail to which they added 2 cases. Gerbie *et al.* [38] reported 8 patients with choriocarcinoma of the ovary treated at Northwestern

University Medical Center. Fewer than half of the reported cases are pure.

Two patients with pure nongestational choriocarcinoma of the ovary have been seen at UT M.D. Anderson Hospital. The first was a 27-year-old white patient who presented with primary amenorrhea, irregular vaginal bleeding and abdominal pain. Nine years prior to referral she had undergone a left salpingo-oophorectomy for an ovarian mass that revealed gonadoblastoma with a focus of dysgerminoma. Investigation revealed an 18-week size pelvic mass, pulmonary metastases on chest x-ray (stage IV), and a serum chorionic gonadotropin level of 935,000 IU per liter. She subsequently underwent exploratory laparotomy with biopsy of pelvic mass arising from the right ovary. Metastatic disease was noted on the liver capsule. Histologic examination revealed a pure choriocarcinoma. The other patient was a white female who presented at age 14 with abdominal pain and a palpable mass. Serum chorionic gonadotropin was 303,000 IU per liter. Dilatation and curettage revealed only decidual changes in the endometrium and exploratory laparotomy revealed a large left ovarian mass with preoperative rupture and hemoperitoneum. The tumor was densely adherent in the cul-de-sac and no other metastatic disease was noted (stage IIc).

The clinical and operative findings in patients with choriocarcinoma are similar to those in patients with other malignant germ cell tumors. The age range of the reported patients has been from 7 months to 37 years. In addition to the usual signs and symptoms, precocious puberty may be predominantly observed in these patients because of the production of chorionic gonadotropin.

Macroscopically this tumor is usually unilateral, solid, gray-white, and hemorrhagic. Necrosis may also be present. Microscopically the essential feature is the presence of both cytotrophoblast and syncytiotrophoblast (Figure 7). The cytotrophoblast is composed of medium-sized polygonal, round, or oval cells with clear cytoplasm and sharp nuclear borders. The syncytiotrophoblast is composed of large or very large basophilic vacuolated cells with irregular outlines. They contain multiple hyperchromatic nuclei varying in size and shape or large masses of chromatin. These form plexiform patterns with central nests of cytotrophoblast enclosed by rims of syncytiotrophoblast. As noted, hemorrhage and necrosis may be prominent.

8. Polyembryoma

Polyembryoma is an exceedingly rare tumor composed of numerous embryoid bodies morphologically resembling embryos. Peyron [39, 40] originally described this neoplasm as a component of a testicular teratoma. Only 8 cases of this ovarian neoplasm have been reported [41–47], most of

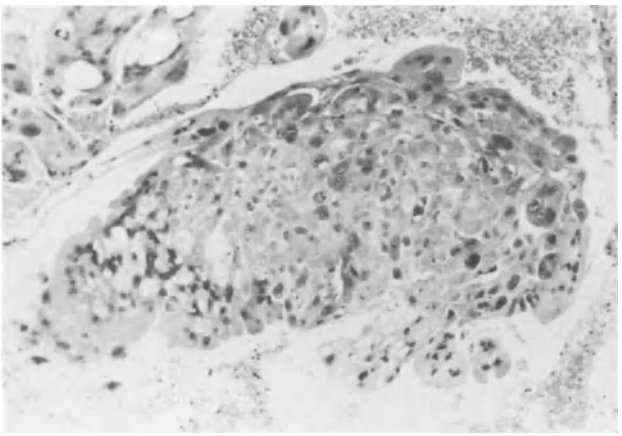

Figure 7. Choriocarcinoma. Typical biphasic pattern composed of mononuclear cytotrophoblasts surrounded by darker staining multinucleated and vacuolated syncytiotrophoblasts (H & E, × 50).

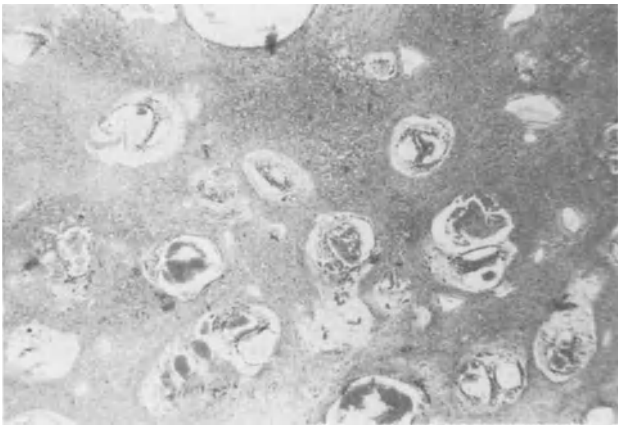

Figure 8. Polyembryoma. This low power view shows multiple embryoid bodies surrounded by collagenous stroma (H & E, × 12.5).

which were components of mixed tumors. The only patient with this tumor seen at UT M.D. Anderson Hospital had a pure polyembryoma and has previously been reported by Beck *et al.* [41] in 1969. She was 26 years old at the time of diagnosis, presenting with a 3-month history of irregular vaginal bleeding and a palpable pelvic mass. Her tumor was confined to the right ovary (stage Iai) which was 10 cm in diameter.

The clinical and operative findings observed in these patients are similar to those in patients with other malignant germ cell tumors. In addition, precocious puberty may be a prominent symptom because of chorionic gonadotropin production by the tumor.

Macroscopically, these tumors are usually unilateral, solid, and contain

hemorrhage and necrosis. Microscopically, they contain numerous embryoid bodies of varying differentiation and size (Figure 8). Trophoblastic differentiation may occasionally be observed.

9. Pathology – pitfalls

In order to continue to accumulate more information about germ cell tumors, thereby making progress in terms of understanding their biologic behavior and improving clinical management and resultant survival, it is imperative that pathologic study of these neoplasms be meticulous and thorough. Because of the rarity of these tumors, most pathologists will see very few such cases in the course of their careers. Ideally, then, if there is any question concerning the diagnosis of a germ cell tumor, pathologic material should be forwarded to a pathologist whose primary field of interest is ovarian cancer. Likewise, with any ovarian specimen from a child or young adult in which there is some question concerning the exact diagnosis, the diagnosis of germ cell tumor should always be suspected and consultation sought if necessary.

In the UT M.D. Anderson series several cases were originally misdiagnosed as being undifferentiated epithelial tumors, benign tumors, or other types of tumors, resulting in delays in treatment, suboptimal clinical management, and at times unnecessary death.

Not only is it important to correctly diagnose an ovarian neoplasm as belonging to the category of germ cell tumors, it is equally important to adequately sample the tumor and meticulously examine it. Sections should be taken for every one centimeter of greatest tumor diameter. Sections should be taken from areas that grossly appear dissimilar. In the past it was common practice to label a tumor based on the predominant component. This practice is no longer acceptable. The number, type, and percentage of various elements within the same tumor must be precisely documented. Pathologic diagnosis must be correlated with clinical findings, including operative description and determination of serum tumor markers. Whenever indicated, immunoperoxidase stains should be performed.

Small inaccuracies in histologic diagnosis may lead to major differences in clinical management decisions. For instance, a patient with a pure dysgerminoma might be treated postoperatively with irradiation therapy under certain circumstances. On the other hand, the presence of even a small amount of endodermal sinus element in the tumor would necessitate combination chemotherapy rather than irradiation therapy. Only by meticulously studying these tumors will we be able to appreciate subtle differences in behavior and prognosis, and thereby make adjustments in clinical management.

10. Associated abnormal sexual development

It is currently well-appreciated that malignant germ cell tumors of the ovary may arise in phenotypic females with gonadal dysgenesis. Most commonly, this condition occurs in a preexisting precursor lesion, the gonadoblastoma. Gonadoblastoma was first reported in 1953 by Scully [48], who described the tumor as composed of germ cells and sex cord stroma. Since that time there have been several reports of malignant germ cell tumors, usually dysgerminomas, arising in patients with dysgenetic gonads [49–61]. The vast majority of these patients, but not all, have a Y-chromosome present. Classic examples of this type of patient include testicular feminization (phenotypic female, 46XY karyotype, androgen insensitivity), pure gonadal dysgenesis (phenotypic female, 46XY or XY mosaic karyotype, normal height), hermaphroditism (46XY or 46XX karyotype), mixed gonadal dysgenesis (phenotypic female, 45XO/46XY mosaic karyotype, with or without stigmata of Turner's syndrome), or Turner's syndrome (phenotypic female, 45XO karyotype). An estimate of the probability of a patient with dysgenetic gonads and a Y-chromosome developing a malignant germ cell tumor is 25–30%. In the present series there were 7 patients with malignant germ cell tumors who had dysgenetic gonads. All had 46XY karyotype. Possibly more went undetected. The management of such lesions will be discussed below.

11. Tumor markers

Among ovarian neoplasms, germ cell tumors possess the unique property, in many cases, of producing biological markers which can be detected in the serum. The development of specific and sensitive radioimmunoassay techniques for measuring hCG and AFP have led to dramatic improvements in the monitoring of patients with germ cell tumors of the ovary. At the present time, serial measurements of these serum markers may aid in the diagnosis of these tumors and may be used to monitor the effects of treatment as well as to detect the presence of subclinical disease recurrence.

Chorionic gonadotropin is a glycoprotein synthesized by trophoblastic tissue. The relationship of hCG to normal gestation was first described by Ascheim and Zondek [62] in 1928. It was soon thereafter appreciated that neoplastic trophoblastic tissue appears to retain the ability to produce hCG. From that point in time until the mid-1960s a number of biological assays for hCG were developed. During this period, it was an accepted fact that use of hCG in monitoring patients with trophoblastic neoplasms who were receiving chemotherapy was hindered by the cross-reactivity of human luteinizing hormone with hCG. In the 1960s the development of radioim-

munoassay techniques allowed measurement of low levels of hCG in the serum [63]. In the 1970s the development of the beta-subunit hCG radioimmunoassay revolutionized the management of trophoblastic neoplasms [64].

Bergstrand and Czar [65] first identified AFP in 1956. Other investigators subsequently demonstrated the presence of AFP in sera of patients with liver cancer [66, 67]. Gitlin *et al.* [68] showed that the human yolk sac is capable of synthesizing AFP. Since that time several biochemical and histochemical studies have confirmed the presence of elevated levels of AFP in patients with endodermal sinus tumor [14, 19–24, 69].

Therefore, only in the last few years have we been able to assimilate and integrate this knowledge in order to optimally incorporate it into the management of patients with germ cell tumors of the ovary. Table 3 illustrates the typical findings in the sera of patients with the various tumor subtypes. Endodermal sinus tumor and choriocarcinoma are the prototypes of AFP and hCG production, respectively. Immunohistochemical studies have demonstrated the presence of these markers in virtually 100% of tumors studied [14, 30]. AFP seems to be localized in the cytoplasm of cells or in hyaline bodies. Chorionic gonadotropin is localized in syncytiotrophoblastic cells. Embryonal carcinoma is capable of producing both hCG and AFP, more commonly the former [29]. The exceedingly rare polyembryoma has been reported as producing hCG [41, 47, 70]. There is at least one report describing the localization of AFP in a pure polyembryoma [47].

Dysgerminoma is commonly considered to be void of any hormonal production. Indeed, most papers on the subject do not even mention tumor markers. On the other hand, multi-nucleated syncytiotrophoblastic giant cells may occasionally be found in dysgerminoma, and may be responsible for hCG production, usually at low levels [71, 72]. Likewise, although immature teratoma is considered to be free of hormonal production, there have been several reports of AFP production by this tumor [22, 73–77]. How many of these reports truly describe pure immature teratoma remains unknown. Only by correlating serum tumor marker results with meticulous

Table 3. Biologic markers in germ cell tumors of the ovary

Histology	AFP	hCG
Dysgerminoma	−	±
Immature teratoma	±	−
Endodermal sinus tumor	+	−
Mixed germ cell tumor	±	±
Choriocarcinoma	−	+
Embryonal carcinoma	±	+
Polyembryoma	±	+

Table 4. Biologic markers in germ cell tumors – UT M.D. Anderson Hospital Series 1944–1983

Histology	No. patients tested	AFP+	hCG+
Dysgerminoma	17/74	0	5
Immature teratoma	15/51	2	1
Endodermal sinus tumor	9/45	9	0
Mixed germ cell tumor	21/42	8	10
Choriocarcinoma	2/2	0	2
Embryonal carcinoma	1/1	–	1
Polyembryoma	1/1	–	1

pathologic study will the true incidence of AFP production by immature teratoma become evident. Mixed tumors, of course, may produce either hCG or AFP, or both, depending on the type and quantity of elements present. Table 4 presents the tumor marker results in the present series.

A third serologic tumor marker that has recently received increasing attention is the glycolytic enzyme, lactic dehydrogenase (LDH). Lippert and Javadpour [78] found that serum LDH levels were elevated in a number of patients with bulky testicular tumors, and at times was the sole elevated tumor marker which could be serially monitored during therapy. Liu *et al.* [79] reported the elevation of an LDH isoenzyme, lactic dehydrogenase – 1, in 81% of patients with stage III testicular cancer.

In 1964 Zondag [80] was apparently the first to recognize elevated serum LDH isoenzymes in patients with dysgerminoma of the ovary. In 1982 Sheiko and Hart [81] reviewed the world literature on the topic – 4 previously reported cases of ovarian dysgerminomas with elevated LDH levels – and reported a fifth case with abnormally high levels of LDH fractions 1, 2, and 3. Awais [82] more recently described 6 cases of ovarian dysgerminomas, 5 of whom had elevated serum LDH prior to therapy which declined to normal levels after treatment. Certainly serologic testing of LDH isoenzymes in ovarian dysgerminomas as well as in nondysgerminomatous ovarian germ cell tumors deserves further attention in the future.

12. Clinical management of dysgerminoma of the ovary

Like other malignant germ cell tumors, the majority of dysgerminoma patients are stage I when diagnosed. Kurman and Norris [15] reported that 75% of dysgerminomas seen at the Armed Forces Institute of Pathology were stage I. The main route of dissemination is by regional lymphatics, most commonly to the paraaortic and iliac lymph nodes. From these areas spread

may occur to mediastinal and supraclavicular lymph nodes. Peritoneal spread may also occur. Hematogenous spread generally occurs only late in the disease process and tends to involve the liver, lungs, or bones most commonly.

Management of dysgerminoma of the ovary should be individualized depending on several factors, including age of the patient, desire for future childbearing, and operative findings. Surgical excision is the obvious initial step in the diagnosis and treatment of this tumor. If time allows (i.e., the patient does not present with acute abdominal pain), preoperative management of a young female with a pelvic mass should include a chest x-ray, intravenous pyelogram, barium enema, routine blood studies, and determination of serum tumor markers – β-hCG, AFP, and LDH isoenzymes. Optional studies, depending on the clinical situation, include sonography and lymphangiography.

Since, as with most germ cell tumors of the ovary, dysgerminomas tend to be quite large, a generous vertical incision should be employed in any patient suspected of having such findings. Especially in a young patient, there is a great tendency among surgeons to utilize a lower transverse abdominal incision for cosmetic reasons. While this motivation is admirable, it may seriously impair exposure to the upper abdomen, including the paraaortic lymph nodes, and may possibly compromise staging of the malignancy leading to potentially fatal errors in future management.

Upon entering the abdomen, if ascites is present, it should be evacuated and sent for cytologic study. If no ascites is present, then saline cytologic washings of the pelvis and bilateral paracolic gutters should be obtained. Following this, one should initially inspect and palpate, if possible, the entire peritoneal contents in a methodical manner, including the structures of the upper abdomen, all peritoneal surfaces (including the diaphragmatic surfaces), the entire small intestine, colon, omentum, retroperitoneal areas, and pelvic organs. Attention should then be turned to the ovarian mass. If the ovarian pathology is unilateral (which it usually is with germ cell tumors), a unilateral salpingo-oophorectomy should be performed, making special effort not to rupture the mass. If bilateral tumors are present, then the more suspicious side should first be separately removed. Frozen section examination should then be performed. If the tumor represents a pure dysgerminoma, then careful inspection and palpation of the contralateral ovary should ensue. Unlike other germ cell tumors, bilaterality of dysgerminoma occurs in approximately 10%–15% of cases [83–86], although it has been reported lower in some series [87, 88], and higher in others [89]. The contralateral ovary should be excised if it clearly contains tumor or if it is unequivocally dysgenetic. Conversely, if the contralateral ovary appears completely normal, it probably should still be removed in the older patient who has definitely completed childbearing. Otherwise, in view of the rela-

tively high incidence of bilaterality, a wedge biopsy with frozen section should be performed. It has been estimated that approximately one-third of contralateral ovarian involvement is occult or microscopic [15]. If bilateral oophorectomy is indicated, then hysterectomy should also be performed in most cases to obviate future potential problems with uterine bleeding or pathology.

Following establishment of a diagnosis of dysgerminoma, any suspicious areas in the remainder of the abdomen or pelvis should be excised or biopsied. Although there is currently no sound scientific basis for maximum cytoreductive surgery in this disease, it is the authors' recommendation that the same principles that have been applied to the surgical management of epithelial ovarian cancer be followed. One must, however, exercise common sense and weigh potential benefit of an aggressive surgical approach against the potential risks or complications. In addition, one must be mindful of the fact that dysgerminoma is an exquisitely radiosensitive tumor.

If meticulous search for extra-ovarian disease yields negative findings, then random biopsies of high-risk sites should be performed, including sampling of the omentum, peritoneal surfaces in the pelvis and upper abdomen, and retroperitoneal lymph nodes, especially paraaortic nodes.

Thorough pathologic sampling and analysis is critical. The presence of other germ cell elements must be excluded. Misdiagnosis of a pure dysgerminoma in the presence of other malignant germ cell elements may prove fatal. Perioperative determination of serum tumor markers will certainly supplement histologic diagnosis. As noted above, however, a small percentage of dysgerminomas will be associated with an elevated serum hCG.

Once the patient has recovered from surgery, postoperative lymphangiography and/or CAT scanning of the abdomen and pelvis should be considered, especially if initial exploration has been inadequate. DePalo *et al.* [86] reported positive lymphangiogram findings in 14 of 44 (31.8%) patients with dysgerminoma referred to his institution. In patients with clinical stage I and II disease, lymphangiography was positive in 31.6% (12/38 patients). Such findings would possibly alter postoperative management.

Earlier reports indicated a rather poor prognosis, with 5-year survival of 27% to 33% [84, 90]. Several factors undoubtedly contributed to these dismal results, including suboptimal therapy, pre-megavoltage irradiation therapy, the presence of several patients with advanced disease, and inclusion of many mixed tumors in these series. More recent studies have reported a much-improved prognosis for pure dysgerminomas, with 5-year survival results in the range of 75%–90% [83, 86–88, 91–97].

Two major factors influence treatment decisions in patients with dysgerminoma – survival and preservation of childbearing capacity. While dysgerminomas are exquisitely radiosensitive, the majority of patients are young and capable of future childbearing if one ovary is preserved. Therefore, the

major therapeutic dilemma in dysgerminoma is whether or not to recommend conservative management for early disease. Following surgery alone consisting of unilateral salpingo-oophorectomy for stage Ia disease, recurrence rates vary from 17%–52.8% [83, 88, 89, 92, 93, 98]. Higher recurrence rates are probably attributable to inadequate staging procedures and inclusion of mixed tumors in many series. With careful initial staging and inclusion of only pure dysgerminomas, recurrence rates following conservative therapy for stage Ia disease should be less than 20%. At UT M.D. Anderson Hospital, 14 patients with stage Iai disease, all but 1 of whom underwent initial surgery elsewhere, have been treated conservatively. Three patients, or 21%, have recurred and subsequently received radiotherapy. All 14 patients are alive without evidence of disease 1–300 months since initial diagnosis. The following criteria should be used to select patients with pure dysgerminoma for conservative therapy: 1) unilateral, non-adherent, encapsulated, unruptured tumor (stage Iai); 2) no ascites or positive cytologic washings; 3) no evidence of extra-ovarian disease at the time of surgery; 4) negative pathology of staging biopsies, including lymph nodes and contralateral ovary; 5) negative postoperative lymphangiogram; 6) desire for future childbearing; 7) agreement to close follow-up. The matter of tumor size is a controversial point. Some authors [96, 99] recommend that only patients with tumors less than 10 cm in diameter should be treated conservatively, stating that larger tumors are more likely to recur. The evidence for this is somewhat questionable, based on only a few cases. Gordon *et al.* [88] found no statistical difference in survival based on tumor size. Further study is necessary to clarify this issue.

All patients who do not meet the criteria for conservative management should be treated with total abdominal hysterectomy and bilateral salpingo-oophorectomy followed by radiotherapy. Also, most patients with recurrent disease would be candidates for radiotherapy. The standard radiotherapy program for dysgerminoma consists of treatment to the whole abdomen in a dose of approximately 2000 rad by either the moving strip technique with a ^{60}Co unit, or with open fields employing a 25-MeV photon beam. An additional 1500 rad is delivered to the pelvis. If paraaortic nodal disease is present as documented at the time of surgery or by lymphangiography, an additional 1000–1500 rad is administered to a paraaortic field. Following a hiatus of 4 weeks, the mediastinum and supraclavicular areas receive 2500 rad over a 3-week period. These are the radiotherapy procedures employed at UT M.D. Anderson Hospital [100]. Radiotherapy techniques may vary from one center to another.

Of those patients who do develop recurrent disease, the great majority do so within 2 years. Asadourian and Taylor [83] reported that 65% of recurrences occurred in the first 2 years following diagnosis, and 95.6% occurred within 5 years. Pedowitz *et al.* [98] reported that 80% of recurrences occur-

red within 2 years after diagnosis. Among patients with stage I disease, 5-year survival rates range from 85%–100% [83, 86–88, 96, 97]. For patients with more advanced disease (stage II–IV), 5-year survival rates range from 53%–90% [83, 86–88, 96, 97]. Of course, these patients were treated in a variety of ways, ranging from conservative surgery alone to definitive surgery followed by radiotherapy. The radiocurability of recurrent disease in stage I patients ranges from 60%–100% [87, 88, 96]. Common sites of recurrence include the contralateral ovary, upper abdomen, pelvis, or retroperitoneal lymph nodes [86, 88, 96]. Less common sites include lung, bone, and skin. Recurrence in previously irradiated fields is unusual.

Challenges for the future in the treatment of dysgerminoma consist of exploring treatment modalities that will improve survival at the same time reproductive function is preserved. There have been a few reports describing the use of chemotherapy for dysgerminoma. Creasman *et al.* [10] reported the use of combination chemotherapy consisting of methotrexate, actinomycin-D, and cyclophosphamide (MAC) following surgery in 5 patients with stage Ia anaplastic dysgerminoma. The surgical treatment in 4 of these patients consisted of unilateral salpingo-oophorectomy only. All 5 patients were surviving without evidence of disease 3–36 months following diagnosis at the time of the report. The use of chemotherapy in the treatment of initial advanced disease or recurrent disease has also been reported [96, 97, 102–104]. In some of these cases, geographic distribution of disease precluded the use of radiotherapy. Krepart *et al.* [96] reported 3 patients who received chemotherapy for recurrent disease. Two of these patients responded to a combination of actinomycin-D, 5-fluorouracil, and cyclophosphamide. The other patient failed to respond to cyclophosphamide. Boyes *et al.* [97] treated 2 patients with recurrent disease with alkylating agents. Both patients achieved a complete remission and were disease-free 6 and 9 years later, respectively. Cohen and Goldsmith [102] reported a prolonged complete remission in 1 patient with metastatic dysgerminoma treated with a combination of vincristine and bleomycin, followed by maintenance therapy with vincristine and methotrexate. Weinblatt and Ortega [103] reported a survival of 10 years in a patient with inoperable stage III dysgerminoma treated with 20 cycles of combination chemotherapy with vincristine, actinomycin-D, and cyclophosphamide. More recently, Newlands *et al.* [104] described 3 patients with metastatic dysgerminoma who achieved a complete remission with sequential combination therapy including vincristine, methotrexate, bleomycin, cis-platinum, etoposide, actinomycin, cyclophosphamide, vinblastine, hydroxyurea, and chlorambucil. Future efforts should concentrate on the use of regimens most active in non-dysgerminomatous germ cell tumors, namely the combination of vincristine, actinomycin-D, and cyclophosphamide, or the combination of vinblastine, bleomycin, and cis-platinum. Consideration should be given to proto-

cols for early disease with preservation of reproductive capability as well as for advanced or recurrent disease. There is certainly a growing body of literature documenting the efficacy of chemotherapy in advanced or metastatic testicular seminoma [105–108]. Cis-platinum or the combination of vinblastine, bleomycin, and cis-platinum seem to be the most commonly employed regimens.

A special clinical problem arises when dysgerminoma is associated with pregnancy. Dysgerminoma constitutes 25%–35% of all ovarian cancers coexisting with pregnancy [109–111]. Conversely, approximately 15%–20% of all dysgerminomas are diagnosed during pregnancy or in the immediate postpartum period [96]. When associated with pregnancy, dysgerminoma may lead to obstetric complications, change in management of the pregnancy, or even fetal demise. When an ovarian mass is identified during the first two trimesters of pregnancy and does not resolve within a reasonable length of time, laparotomy with resection is generally indicated. In the third trimester, some delay is usually acceptable until fetal maturity is achieved, at which time laparotomy with surgical excision and cesarean section may be performed. If extraovarian spread of dysgerminoma is diagnosed at laparotomy, then the generally accepted management would be to proceed with hysterectomy and bilateral salpingo-oophorectomy with termination of the pregnancy. Postoperatively radiotherapy should be administered. With stage Ia disease, if the above criteria for conservative therapy are met, then a unilateral salpingo-oophorectomy with proper staging biopsies and cytologies is acceptable, although somewhat controversial. Karlen *et al.* [112] have presented a rather comprehensive review of the management of dysgerminoma associated with pregnancy.

In summary, the prognosis for dysgerminoma is generally excellent with adequate staging procedures, appropriate surgical management, and postoperative radiotherapy, when indicated. Whenever optimal therapy guidelines are followed, recurrence rates following conservative therapy for early disease will hopefully be much less than those reported in the literature.

13. Clinical management of nondysgerminomatous germ cell tumors of the ovary

Nondysgerminomatous germ cell tumors of the ovary include endodermal sinus tumor, immature teratoma, embryonal carcinoma, nongestational choriocarcinoma, polyembryoma, and mixed germ cell tumors. As noted above, the great majority of these tumors are stage I when diagnosed, with stage III being the next most common stage. Like dysgerminoma, they have a greater propensity than epithelial tumors to disseminate via lymphatics, although peritoneal spread is also common. Hematogenous spread is usually

noted late in the course of progressive disease, with liver or lung involvement. Conversely, unlike dysgerminoma, they are radioresistant and have a much lower incidence of true bilaterality. With our present state of knowledge, the treatment of these tumors is basically the same, with some minor or subtle differences, which will be discussed below. Basing therapeutic decision-making on available literature is greatly hindered by several factors: 1) many of the classical papers describing the largest series mainly include patients treated prior to the modern combination chemotherapy era; 2) nomenclature and categorization of these tumors is confusing; 3) clinical data, mainly FIGO staging of these tumors, historically has been grossly inadequate; 4) pathologic data in many reports is inadequate and misleading, with tumors labelled according to the predominant component only, thereby including mixed tumors in supposedly pure series; 5) because of the rarity of these tumors, few large series describing modern postoperative treatment exist, with mainly case reports or reports of small numbers of patients. Indeed, prospective randomized clinical trials attempting to answer subtle, yet important, questions concerning modern treatment are virtually nonexistent. Despite all these obstacles, however, a tremendous amount of progress has occurred over the last 2 decades, transforming an almost uniformly fatal disease into one with an excellent prognosis in the great majority of cases.

Individualization in the treatment of these tumors is also possible, based on patient age, desire for preservation of reproductive capacity, and operative findings. In a young female with a pelvic mass and any of the other common signs and symptoms, surgery is the first step in diagnosis and treatment, unless, of course, a benign functional cyst is strongly suspected. In the latter case, a short period of observation may be prudent in order to see if the mass spontaneously resolves. This judgment must be based on the sum of clinical findings, including severity and type of signs and symptoms, physical examination findings (including size and consistency of the mass), and indicated laboratory data, including measurement of serum tumor markers. If surgery is indicated and time allows (i.e., acute abdomen not present), then preoperative studies should include a chest x-ray, intravenous pyelogram, barium enema, routine blood studies, and determination of serum tumor markers – β-hCG, AFP and LDH isoenzymes. Optional studies, depending on the clinical situation, would include sonography and lymphangiography.

At surgery, a generous vertical incision should be employed. Although cosmetically superior, a transverse incision generally impairs exposure to the upper abdomen, especially the paraaortic lymph node region, and thereby potentially leads to inadequate staging and consequent errors in postoperative therapy.

Upon entering the abdomen, if ascites is present, it should be evacuated

and forwarded for cytologic analysis. If no ascites is present, then saline cytologic washings of the pelvis and paracolic gutters should be obtained. Following this, one should initially inspect and palpate, if possible, the entire peritoneal contents in a methodical manner, including upper abdominal structures, all peritoneal surfaces (including diaphragmatic surfaces), the entire small intestine, colon, omentum, retroperitoneal areas, and pelvic organs. Once completed, attention should turn toward the ovarian mass. If ovarian pathology is unilateral (as is usually the case with these tumors), then unilateral salpingo-oophorectomy should be performed, making special effort not to rupture the mass. If bilateral tumors are present, then the more suspicious side should initially be excised. If frozen section examination reveals a malignant nondysgerminomatous germ cell tumor, then the contralateral ovary should be carefully examined. As noted, bilaterality of these tumors is less common than in dysgerminoma, although it may occur. Moreover, occult contralateral ovarian involvement must be exceedingly rare. In the present series, there were no patients with nondysgerminamatous germ cell tumors with documented occult contralateral ovarian disease. In the AFIP series [15], of 35 contralateral grossly normal ovaries examined microscopically, only 1 was positive for occult tumor, that being a mixed germ cell tumor containing dysgerminoma, the sole element in the occultly positive ovary. Therefore, although biopsy of a grossly normal contralateral ovary remains controversial, it is the authors' recommendation to forego random biopsy which may theoretically lead to infertility secondary to peritoneal adhesions or ovarian failure. In the present series, there were only 3 stage Ib tumors, all mixed tumors.

If the contralateral ovary is abnormally enlarged, statistically it more likely represents a benign cystic teratoma. In such a case, ovarian cystectomy with ovarian preservation may be accomplished. If, on the other hand, the contralateral ovary obviously contains tumor or is unequivocally dysgenetic, then it should be excised. Bilateral salpingo-oophorectomy should also be considered in the rare older patient (over age 40) who has completed childbearing. If bilateral adnexectomy is performed, then hysterectomy should also be accomplished in most cases to avoid future potential problems with uterine bleeding or pathology.

Even in the face of extensive disease, one may occasionally be able to preserve a normal contralateral ovary. Any suspicious other areas should be excised or biopsied. The concept of maximum cytoreductive surgery for these tumors, although not scientifically supported, seems to be reasonable. One must, however, exercise common sense and sound surgical judgement, weighing benefits of aggressive surgery against potential risks or complications.

If no obvious extra-ovarian disease is present, then random staging biopsies of high-risk areas should be performed, including sampling of the

omentum, peritoneal surfaces in the pelvis and upper abdomen, and retroperitoneal lymph nodes, especially paraaortic nodes.

Inadequate incision and sub-standard staging procedures remain problem areas in the surgical treatment of these tumors. In addition, overtreatment with unnecessary bilateral salpingo-oophorectomy continues to be practiced by those who do not fully understand this disease. Several studies have confirmed the fact that unilateral adnexectomy compared to bilateral extirpation does not adversely influence survival in stage I disease [14, 26, 29, 33, 34, 38, 39, 113].

As with dysgerminoma, thorough pathologic sampling and analysis is of critical importance. The type of elements present and estimated percentage of each should be noted. Serum tumor marker levels will help supplement or confirm histologic data in many cases.

Immediate postoperative management should include determination of serum tumor marker levels, as well as consideration for lymphangiography and computerized tomography of the abdomen and pelvis, if not performed in the preoperative period, especially if initial staging procedures have been inadequate. If tumor markers are found to be elevated, they should be monitored at monthly intervals for a minimum of 1 year, and possibly, for 2 years following initial diagnosis. This will serve 2 purposes – to assist in monitoring response to therapy, and to detect subclinical recurrence, if it occurs, following completion of treatment. Determination of chromosomal karyotype should be performed in any patient with suspicious findings compatible with dysgenetic gonads.

The postoperative treatment of nondysgerminomatous germ cell tumors has varied considerably over the last several years. From the 1940s until at least the mid-1960s, it was common practice to treat such patients with surgery alone, or to administer postoperative radiotherapy or radioisotope therapy. Beginning in the late 1950s, some patients received postoperative treatment with single akylating agent therapy. Since the mid-1960s, a variety of combination chemotherapy regimens have been employed, with much-improved results. The results of the present series include 135 evaluable patients with nondysgerminomatous germ cell tumors (7 patients are currently undergoing treatment and are excluded from treatment and survival analyses).

Several reports have consistently documented the dismal survival associated with surgery alone. Describing the AFIP series, Kurman and Norris [14] reported 27 patients with stage I endodermal sinus tumor treated with surgery alone (18 with unilateral salpingo-oophorectomy, 9 with hysterectomy and bilateral salpingo-oophorectomy). Five of these patients, or 19%, were alive and well. Jimerson and Woodruff [25] reported 22 patients with stage I endodermal sinus tumor treated with surgery alone, 2 of whom were surviving. Gallion *et al.* [114], reviewing the available literature (in-

cluding the above 2 references), found a total of 51 patients with endodermal sinus tumor treated with surgery alone, 7 of whom (14%) were surviving at 2 years. Norris *et al.* [33] also noted that of 40 patients with stage I immature teratoma treated with surgery alone, 10 patients had died at the time of the report; 4 additional patients had developed recurrent disease, but were surviving, 3 with tumor. It should be noted that all 14 of the patients with grade 1 lesions survived, although 1 patient recurred at 8 months and underwent a second surgical procedure. Fourteen patients with stage I mixed germ cell tumors of the ovary reported from the AFIP [34] underwent surgery alone as initial treatment; only 7 were surviving at the time of the report. Of 11 patients with stage I mixed tumors treated with surgery alone reported from the Emil Novak Ovarian Tumor Registry [115], only 2 survived.

Table 5 shows the survival results of patients in the UT M.D. Anderson series treated initially with surgery alone. Of the 33 patients who initially were treated with surgery alone, only 3 patients (1 endodermal sinus tumor, 2 immature teratoma) are surviving without evidence of disease without any further therapy. Of the remaining 30 patients, all developed recurrent disease; 11 patients are surviving, having been salvaged with subsequent therapy following recurrence. Of the 11 patients who were cured following recurrence, 4 patients received vincristine, actinomycin-D, and cyclophosphamide (VAC), 1 received vinblastine, bleomycin, and cis-platinum (VBP), 5 received a combination of VAC and VBP, and 1 patient received VAC followed by Adriamycin and cyclophosphamide. Therefore, based on the above data, the only type of patient with nondysgerminomatous germ cell tumor who should even be considered for treatment with surgery alone would be the well-staged patient with stage Iai grade 1 immature teratoma. Possibly, even in these patients, a brief course of adjuvant chemotherapy should be considered.

Table 5. Survival by postoperative treatment surgery alone

	Stage				
Histology	I	II	III	IV	Total
Immature teratoma	8/14	0/1	4/6	–	12/21
Endodermal sinus tumor	1/2	–	0/2	–	1/4
Mixed germ cell tumor	0/6	–	1/1	0/1	1/8
Choriocarcinoma	–	–	–	–	0
Embryonal carcinoma	–	–	–	–	0
Polyembryoma	–	–	–	–	0
Total	9/22	0/1	5/9	0/1	14/33
Percent	41	0	56	0	44

Equally dismal survival has resulted from postoperative treatment with radioisotopes, radiotherapy, or single alkylating agent therapy. Kurman and Norris [14] reported 20 patients with endodermal sinus tumor, all of whom received postoperative treatment with external radiation, radioactive colloidal gold (^{198}Au), or radioactive chromic phosphate (^{32}P). None survived. Of 4 patients with stage I disease who received postoperative alkylating agents, all are dead. Jimerson and Woodruff [25] reported 5 patients with endodermal sinus tumor who received postoperative radiotherapy, none of whom survived. The only patient treated with postoperative alkylating agent therapy also died. Also from the AFIP series [33], 16 patients with immature teratoma received treatment either following surgery or for recurrence with either radiotherapy, intraperitoneal radioisotopes, chemotherapy, or combinations thereof. Five of these patients survived. Kurman and Norris [34] also reported 8 patients with mixed germ cell tumors who received postoperative external radiation or radioisotopes, 3 of whom survived. Likewise, 2 of 5 patients with embryonal carcinoma treated with postoperative radiotherapy survived in the AFIP series [29].

In the UT M.D. Anderson series, both patients with endodermal sinus tumor who received postoperative instillation of intraperitoneal gold (^{198}Au) died, despite the fact that one patient had a brief response to combination chemotherapy with actinomycin-D, 5-fluorouracil, and cyclophosphamide when recurrence was diagnosed. Similarly, both patients treated with single alkylating agent therapy postoperatively failed treatment, although 1 of these survived following salvage therapy with a combination of vincristine, actinomycin-D, and cyclophosphamide.

Tables 6 and 7 show survival in patients who received initial postoperative treatment with radiotherapy or a combination of radiotherapy and chemotherapy, respectively. Only 2 of 11 patients who received initial posto-

Table 6. Survival by postoperative treatment radiotherapy

	Stage				
Histology	I	II	III	IV	Total
Immature teratoma	–	0/1	0/2	–	0/3
Endodermal sinus tumor	–	–	0/1	–	0/1
Mixed germ cell tumor	0/2	1/2	0/2	–	1/6
Choriocarcinoma	–	–	–	–	0
Embryonal carcinoma	–	–	–	–	0
Polyembryoma	1/1	–	–	–	1/1
Total	1/3	1/3	0/5	0	2/11
Percent	33	33	0	0	18

Table 7. Survival by postoperative treatment radiotherapy+chemotherapy

Histology	Stage				Total
	I	II	III	IV	
Immature teratoma	0/1	–	–	0/1	0/2
Endodermal sinus tumor	0/1	0/1	–	–	0/2
Mixed germ cell tumor	1/1	–	–	–	1/1
Choriocarcinoma	–	–	–	–	0
Embryonal carcinoma	–	–	–	–	0
Polyembryoma	–	–	–	–	0
Total	1/3	0/1	0	0/1	1/5
Percent	33	0	0	0	20

perative radiotherapy survived, despite the fact that many of these patients received subsequent combination chemotherapy at the time of recurrence. The 2 survivors included one patient with stage Ia polyembryoma and another patients with stage IIb mixed germ cell tumor who recurred following radiotherapy, and then received 2 years of therapy with vincristine, actinomycin-D, and cyclophosphamide. Likewise, only 1 of 5 patients who initially were treated with radiotherapy and chemotherapy survived, that patient being treated with abdominal-pelvic radiation followed immediately by combination chemotherapy with vincristine, actinomycin-D, and cyclophosphamide for a period of 2 years. Therefore, although a few patients are cured by surgery followed by radiotherapy, radioisotopes, or alkylating agents, these postoperative therapies are quite inadequate. There seems to be no role for these therapies in the treatment of nondysgerminomatous germ cell tumors.

Beginning in the mid-1960s, combination chemotherapy was used in the treatment of nondysgerminomatous germ cell tumors. One of the earliest regimens administered was a combination of actinomycin-D, 5-fluorouracil, and cyclophosphamide (AcFuCy). There have since been scattered reports of responses and prolonged survival in a few patients who received AcFuCy [26, 28, 114, 116, 117]. Although there was modest success with AcFuCy, its popularity was short-lived, and it was replaced by other combination regimens.

The combination of methotrexate, actinomycin-D, and cyclophosphamide or chorambucil (MAC) was also used in the early combination chemotherapy era, and is still advocated by some clinicians today. MAC has primarily been administered to patients with choriocarcinoma of the ovary – either gestational, nongestational, or as a component of mixed germ cell tumors [38, 70, 119]. Wider *et al.* [118] reported sustained remissions in 3 of 4 patients with choriocarcinoma of the ovary treated with MAC.

Goldstein and Piro [119] later reported the use of MAC in 11 patients with choriocarcinoma of the ovary or testis, 5 of whom achieved a sustained remission. Gerbie *et al.* [38] described prolonged survival in 2 of 5 patients with choriocarcinoma treated with this regimen. Creasman *et al.* [101] have advocated the use of MAC in all patients with malignant germ cell tumors, reporting an 89% 2-year survival in 26 patients treated with relatively short-term therapy.

Tables 8 and 9 present the experience at UT M.D. Anderson Hospital with AcFuCy and MAC, respectively. Two of 5 patients who received MAC have survived – 1 patient with stage IIc pure choriocarcinoma, and 1 patient with stage Ia mixed germ cell tumor with a major choriocarcinoma component. Four of 7 patients treated with AcFuCy survived. At UT M.D. Anderson Hospital AcFuCy was rather rapidly replaced by the combination of vincristine, actinomycin-D, and cyclophosphamide.

Table 8. Survival by postoperative treatment AcFuCy

	Stage				
Histology	I	II	III	IV	Total
Immature teratoma	2/2	–	–	–	2/2
Endodermal sinus tumor	1/3	–	1/2	–	2/5
Mixed germ cell tumor	–	–	–	–	0
Choriocarcinoma	–	–	–	–	0
Embryonal carcinoma	–	–	–	–	0
Polyembryoma	–	–	–	–	0
Total	3/5	0	1/2	0	4/7
Percent	60	0	50	0	57

Table 9. Survival by postoperative treatment MAC

	Stage				
Histology	I	II	III	IV	Total
Immature teratoma	–	–	–	–	0
Endodermal sinus tumor	–	–	0/1	–	0/1
Mixed germ cell tumor	1/1	–	–	0/1	1/2
Choriocarcinoma	–	1/1	–	0/1	1/2
Embryonal carcinoma	–	–	–	–	0
Polyembryoma	–	–	–	–	0
Total	1/1	1/1	0/1	0/2	2/5
Percent	100	100	0	0	40

Since about 1970 several patients with nondysgerminomatous germ cell tumors have been treated with the combination of vincristine, actinomycin-D, and cyclophosphamide (VAC). Several reports have documented improved survival [26, 28, 120–122]. One of the earliest reports of the use of VAC for germ cell tumors of the ovary was that of Smith and Rutledge [28]. They treated 20 patients with 'embryonal carcinoma' with VAC, with 15 patients surviving at the time of the report. Slayton *et al.* [120] found that 16 of 27 patients, or 58%, of patients with malignant germ cell tumors who were treated with VAC were alive and well.

Table 10 shows the results to date with VAC at UT M.D. Anderson Hospital. Forty-eight of 64 patients, or 75%, who received VAC have survived – 86% of patients with early disease (stage I) and 59% of patients with advanced disease (stages II–IV). Of the 48 patients who survived, 44 were cured with this therapy alone. Four patients failed initial therapy with VAC, but were salvaged with a combination of vinblastine, bleomycin, and cis-platinum, sometimes combined with aggressive surgery. Of the 16 patients who died following initial postoperative VAC therapy, 6 received no further therapy. Ten patients, however, received second-line therapies prior to death – 6 patients failed subsequent therapy with a combination of vinblastine and bleomycin; 1 patient failed secondary therapy with vinblastine, bleomycin, and cis-platinum; and 3 patients failed other miscellaneous second-line treatments.

Since the late 1970s, based on the reports of Samuels *et al.* [123 and Einhorn and Donahue [124], on the use of vinblastine, bleomycin, and cis-platinum (VBP) in testicular cancer, this combination has been employed with increasing frequency in patients with malignant germ cell tumors of the ovary. There are now several reports in the literature of excellent results with the use of VBP [125–130]. Not only does this regimen seem to provide

Table 10. Survival by postoperative treatment VAC

	Stage				
Histology	I	II	III	IV	Total
Immature teratoma	10/12	1/2	8/9	–	19/23
Endodermal sinus tumor	13/14	2/4	2/6	–	17/24
Mixed germ cell tumor	9/11	–	1/3	1/2	11/16
Choriocarcinoma	–	–	–	–	0
Embryonal carcinoma	–	–	1/1	–	1/1
Polyembryoma	–	–	–	–	0
Total	32/37	3/6	12/19	1/2	48/64
Percent	86	50	63	50	75

excellent results as front-line therapy, it seems to be effective in salvaging patients who have failed other therapies in up to 50% of cases [127, 128, 130].

In the UT M.D. Anderson Hospital series, 2 patients – 1 patient with stage Ic endodermal sinus tumor, and 1 patient with stage IIc mixed germ cell tumor – have been successfully treated with 6 cycles of VBP. Another 4 patients with advanced mixed germ cell tumors are currently under treatment with this regimen. Moreover, as discussed above, patients who fail other treatment may be salvaged with VBP. Of patients who developed recurrent disease following treatment with surgery alone, 1 patient was salvaged with VBP and 5 patients were salvaged with sequential combinations of VAC and VBP. Four of 5 patients who failed initial VAC therapy have survived following VBP treatment.

Four patients in the present series received a variety of miscellaneous postoperative treatments (Table 11). Of the 2 survivors, 1 patient with stage Ic endodermal sinus tumor received 8 cycles of vincristine, Adriamycin, and cyclophosphamide, and was then placed on VAC for a total of 2 years of treatment. The other patient had stage III endodermal sinus tumor and received 4 cycles of vinblastine, Adriamycin, and bleomycin.

The overall survival of 135 patients with nondysgerminomatous germ cell tumors is presented in Table 12. Survival time was calculated from the time of diagnosis until time of death or to July 1, 1983, whichever came first. Median survival times were computed using Berkson-Gage life table methods [131].

Details of commonly-used combination chemotherapy regimens are listed in Table 13. These dosages are generally used for normal-size adults, and

Table 11. Survival by postoperative treatment miscellaneous

	Stage				
Histology	I	II	III	IV	Total
Immature teratoma	–	0/1[1]	0/1[2]	–	0/2
Endodermal sinus tumor	1/1[3]	–	1/1[4]	–	2/2
Mixed germ cell tumor	–	–	–	–	0
Choriocarcinoma	–	–	–	–	0
Embryonal carcinoma	–	–	–	–	0
Polyembryoma	–	–	–	–	0
Total	1/1	0/1	1/2	0	2/4

[1] Adriamycin/cyclophosphamide.
[2] Vincristine.
[3] Vincristine/Adriamycin/cyclophosphamide.
[4] Vincristine/Adriamycin/bleomycin.

Table 12. Nondysgerminomatous germ cell tumors - overall survival

Histology	Survival	%	Median survivaltime (mos.)
Immature teratoma	33/51	65	120+
Endodermal sinus tumor	23/43	53	120+
Mixed germ cell tumor	17/37	46	29.6
Choriocarcinoma	1/2	50	1,26+
Embryonal carcinoma	1/1	50	107+
Polyembryoma	1/1	100	195+
Total	76/135	56	–

may need to be modified when administered to smaller pediatric patients or obese adults. There are certainly variations in dosages and scheduling in clinical practice. Until only recently, at UT M.D. Anderson Hospital vincristine in the VAC regimen was administered weekly for 10 to 12 doses instead of monthly. This resulted in unacceptable severe neurotoxicity in some patients. Many clinicians administer bleomycin in the VBP regimen on a weekly basis instead of by continuous infusion. The rationale for continuous infusion bleomycin is based upon data that the half-life is short (less than 2 hours), tissue inactivation is rapid, and the drug is a cell-cycle specific drug acting at the G_2-M interphase [132]. Nevertheless, no firm data exist demonstrating the superiority of continuous infusion over intermittent schedules. The doses and scheduling of vinblastine and cis-platinum may also vary somewhat from one institution to another.

Table 13. Combination chemotherapy for nondysgerminomatous germ cell tumors of ovary

	Regimen*	
VAC		
Vincristine	1–1.5 mg/m^2 on day 1	
Actinomycin-D	0.5 mg/day × 5 days every 4 weeks	
Cyclophosphamide	5–7 mg/kg/day × 5 days every 4 weeks	
VBP		
Vinblastine	0.3 mg/kg in divided doses, days 1 and 2	every 3–4 weeks
Bleomycin	15 mg × 5 days by continuous infusion	
Cis-platinum	100 mg/m^2 on day 1	
MAC		
Methotrexate	15 mg/day × 5 days	every 3–4 weeks
Actinomycin-D	0.5 mg/day × 5 days	
Cyclophosphamide	200–300 mg/day × 5 days	

* All doses given intravenously.

While it is quite evident that combination chemotherapy represents optimal postoperative treatment for patients with nondysgerminomatous germ cell tumors, many questions remain unanswered – choice of chemotherapy regimen, duration of therapy, individualization of therapy, and role of second-look laparotomy.

It remains unclear as to which combination regimen is superior – VAC, VBP, or MAC. Prospects for prospective randomized clinical trials are hampered by the rarity of these neoplasms. At present, none exists. Therefore, choice of therapy should be based on available data on efficacy and toxicity. Our greatest experience has obviously been with VAC, and it seems to be very effective in early disease. On the other hand, VBP also appears to provide excellent, if not better, results in early disease. Therefore, until more experience with VBP accumulates, other considerations must enter into drug choice. The toxicity of VAC, as presently administered, is quite acceptable. Although neutropenia may occur, it is generally mild. No drug-related deaths have occurred in patients who received VAC in this series. The combination of VBP seems to have greater potential for toxicity – both short-term and long-term. The incidence of severe neutropenia and concurrent sepsis is high, requiring frequent hospitalizations for antibiotic treatment. Although bleomycin pulmonary toxicity and cis-platinum nephrotoxicity are rare, the occurrence of either side effect in a young patient with potentially curable disease is devastating. Furthermore, drug-related deaths with VBP are certainly reported [127]. The use of VBP must be accompanied by meticulous monitoring of toxicity and aggressive support whenever necessary. In addition to frequent blood counts, periodic monitoring of pulmonary function and renal function should be performed.

As noted above, the survival rate in patients with advanced disease who receive VAC is approximately 50%, which is certainly disappointing. Preliminary results with VBP in advanced disease seem to be comparable, if not better. At the present time, all patients referred to UT M.D. Anderson Hospital with stages II–IV nondysgerminomatous germ cell tumors are being treated with VBP.

The role of the MAC regimen is even less clear. Although MAC has been advocated for all patients with malignant germ cell tumors [101], this recommendation has not met with overwhelming acceptance to date. Based on present evidence, a more reasonable approach would be to propose that MAC be administered to all patients with pure nongestational choriocarcinoma, and possibly also to those patients with mixed tumors in which choriocarcinoma constitutes the predominant element. On the other hand, there is no evidence to suggest that MAC is superior in efficacy to either VBP or VAC.

Optimal duration of therapy is also unclear. During much of the study period VAC was administered for 24 cycles, or approximately 2 years. Our

current practice is to administer either 12 cycles of VAC or 6 cycles of VBP. Other investigators recommend shorter courses of therapy, e.g., 3–4 cycles of VBP or MAC. If tumor markers are initially elevated, serum levels may serve as a guide in determining duration of therapy. Otherwise, duration of therapy is somewhat artibrary and empirical, based on cumulative experience. In the future, efforts will undoubtedly be made to abbreviate duration of treatment. Such efforts, however, should be undertaken with great caution.

While second-look laparotomy has been employed extensively at UT M.D. Anderson Hospital in patients with nondysgerminomatous germ cell tumors, and is currently included in treatment programs, it may be rapidly becoming obsolete. Table 14 presents second-look laparotomy findings in the present series. As noted, 51 of 52 patients had negative results. The 1 positive outcome was performed after only 6 cycles of VAC, that patients ultimately being cured after further treatment with VBP. Of the 51 patients with negative findings, 2 patients recurred and ultimately died.

It should be noted that 12 patients with negative findings actually had mature glial tissue present. This phenomenon of tissue maturation or retroconversion of immature teratoma with time or therapy has been well-described [133]. Furthermore, as with testicular cancer, masses entirely consisting of mature teratoma in previously treated patients may masquerade as recurrent malignancy.

Based on above findings, one can certainly make a case for discontinuing second-look laparotomy in this group of patients, especially those whose serum tumor markers can be monitored. Again, no prospective randomized studies concerning this aspect exist. Therefore, current practice will have to be based on available evidence and personal preference.

The efficacy of salvage therapy is variable. As noted above, some patients who recur following inadequate treatment may be salvaged by combination

Table 14. Second-look laparotomy findings

Histology	No. second looks	Negative findings	No. surviving
Immature teratoma	25	25[1]	24
Endodermal sinus tumor	15	14	14
Mixed germ cell tumor	11	11[2]	11
Choriocarcinoma	0	–	–
Embryonal carcinoma	1	1	1
Polyembryoma	0	–	–
Total	52	51	50

[1] 10 patients with mature glia at second-look.

[2] 2 patients with mature glia at second-look.

chemotherapy. In addition, approximately 50% of patients who initially fail VAC may be salvaged with VBP. Conversely, those patients who fail VBP may have a dismal prognosis. To date no acceptable salvage regimen has been found for these patients. While etoposide (VP-16) seems to possess some activity against malignant germ cell tumors, our preliminary experience tends to indicate that etopside-containing combinations are unable to induce complete remissions in patients who have failed VBP. More experience with etoposide is needed to clarify this matter.

In rare instances, there may be a role for selective, aggressive surgery in patients who achieve a partial remission with chemotherapy, but who have residual tumor in a single localized area, e.g., the lung or liver parenchyma. Solitary localization prior to attempt at surgical excision should be confirmed by a thorough radiographic search for disseminated disease. Computerized tomography and arteriography are generally optimal studies for such purposes.

In summary, optimal management of nondysgerminomatous germ cell tumors should consist of initial surgery with careful staging, meticulous pathologic analysis, and postoperative combination chemotherapy. It should also be emphasized that postoperative therapy should be instituted promptly following surgery, i.e., within 7–14 days if possible, since these neoplasms are relatively rapidly-growing. Serum tumor markers should be monitored at monthly intervals both during and following completion of therapy for a period of 1 to 2 years. Meticulous attention must be paid to monitoring of side effects, both acute and chronic. By following the above guidelines, most patients should have an excellent outcome. Future prospects should include improving results in advanced disease, continuing the search for better chemotherapeutic agents, finding ways of making treatment more palatable, and refining our understanding of these fascinating neoplasms.

14. Follow-up – successes and sequelae

Following completion of therapy, all patients with malignant germ cell tumors should be closely monitored. The present policy at UT M.D. Anderson Hospital is to evaluate patients at monthly intervals with physical examination and determination of serum tumor markers, whenever present, for a period of at least 1 year. Periodic radiographic studies, including chest x-ray, sonography, lymphangiography, and computerized tomography, should be performed as indicated. Whenever recurrent disease is suspected, appropriate studies should be ordered and appropriate treatment instituted, if indicated.

In addition to surveillance for recurrent neoplasm, patients should be

monitored for chronic therapy side effects. Periodic determination of blood profile, renal function, liver function, and possibly pulmonary function should be considered. Potential untoward long-term effects of commonly-employed drugs in these patients are largely unknown, e.g., long-term effects of cis-platinum on auditory and renal function. The incidence of blood dyscrasias in treated patients is also unclear. In the present series, of 216 treated patients, 1 patient has developed preleukemia 2 years following completion of 12 cycles of VAC for stage IIc grade 3 immature teratoma of the ovary.

The effects of chemotherapy on menstrual function and reproductive capacity have been well-described. Several reports have documented menstrual dysfunction or gonadal failure in young patients treated with chemotherapy or radiotherapy, although the incidence is variable [134–138]. Many patients have or regain normal gonadal function following discontinuation of therapy. In the present series, although no extensive study of menstrual function has been performed to date, it is the authors' impression that most patients with ovarian preservation who survive following combination chemotherapy resume normal menstrual function.

Several successful pregnancies following completion of combination chemotherapy have been reported [134, 139–141]. In the present series, there have been 17 normal full-term pregnancies and deliveries following treatment for malignant germ cell tumor of the ovary, 11 of which have occurred following completion of combination chemotherapy.

References

1. Weiss NS, Homonchuk T, Young JL: Incidence of the histologic type of ovarian cancer: the U.S. third national cancer survey, 1969-1971. Gynecol Oncol 5:161–167, 1977.
2. Abell MR, Johnson VJ, Holtz F: Ovarian neoplasms in childhood and adolescence I. – Tumors of germ cell origin. Am J Obstet Gynecol 92:1059–1081, 1965.
3. Norris HJ, Jensen RD: Relative frequency of ovarian neoplasms in children and adolescents. Cancer 30:713–719, 1972.
4. Teilum G: Classification of endodermal sinus tumour (mesoblastoma vitellinum) and so-called 'embryonal carcinoma' of the ovary. Acta Pathol Microbiol cand 64:407–429, 1965.
5. Gillman J: The development of the gonads in man with consideration of the role of fetal endocrines and the histogenesis of ovarian tumors. Contrib Embryol 32:83–131, 1948.
6. Witschi E: Migration of the germ cells of human embryos from the yolk sac to the primitive gonadal folds. Contrib Embryol 32:69–80, 1948.
7. Teilum G: Homologous tumours in ovary and testis: contribution to classification of gonadal tumours. Acta Obstet Gynecol Scand 24:480–503, 1944.
8. Teilum G: Gonocytoma: homologous ovarian and testicular tumours: 1: with discussion of 'mesonephroma ovarii' (Schiller: Amer J Cancer 1939). Acta Pathol Microbiol Scand 23:242–251, 1946.
9. Friedman NB, Moore RA: Tumors of the testis. A report of 922 cases. Mil Surgeon 99:573–593, 1946.

10. Dixon FJ, Moore RA: Tumors of the Male Sex Organs. Atlas of Tumor Pathology, Sect. VIII, Fasc. 31b and 32. Washington, D.C., Armed Forces Institute of Pathology, 1952.
11. Serov SF, Scully Re, Sobin LH: Histological typing of ovarian tumors. International histological classification of tumors, No. 9. Geneva, World Health Organization, 1973.
12. Chenot M: Contribution à l'étude des épithélimoas primitifs de l'ovaire. Thesis, Paris, 1911.
13. Meyer R: The pathology of some special ovarian tumors and their relation to sex characteristics. Am J Obstet Gynecol 22:697–713, 1931.
14. Kurman RJ, Norris HJ: Endodermal sinus tumor of the ovary. A clinical and pathologic analysis of 71 cases. Cancer 38:2404–2419, 1976.
15. Kurman RJ, Norris HJ: Malignant germ cell tumors of the ovary. Hum Pathol 8:551–564, 1977.
16. Schiller W: Mesonephroma ovarii. Am J Cancer 35:1–21, 1939.
17. Teilum G: Endodermal sinus tumors of the ovary and testis, comparative morphogenesis of the so-called mesonephroma ovarii (Schiller) and extraembryonic (Yolk-sac-allantoic) structures of the rat's placenta. Cancer 12:1092–1105, 1959.
18. Teilum G: The concept of endodermal sinus (yolk sac) tumor. Scand J Immunol 8:75–89 (Suppl), 1978.
19. Ballas M: Yolk sac carcinoma of ovary with alpha fetoprotein in the serum and ascitic fluid demonstrated by immunoosmophoresis. Am J Clin Pathol 57:511–516, 1972.
20. Norgaard-Pedersen B, Albrechtesen R, Teilum G: Serum alpha-fetoprotein as a marker for endodermal sinus tumor (yolk sac tumor) or a vitelline component of teratocarcinoma. Acta Pathol microbiol Scand (A) 83:573–589, 1975.
21. Itoh T, Shirai T, Naka A, *et al.*: Yolk tumor and α-fetoprotein. Clinico-pathological study of four cases. Gan 65:215–226, 1974.
22. Talerman A, Haije WG: Alpha-fetoprotein and germ cell tumors: A possible role of yolk sac tumor in production of alpha-fetoprotein. Cancer 34:1722–1726, 1974.
23. Tsuchida Y, Saito S, Ishida M, *et al.*: Yolk sac tumor and alpha-fetoprotein. A report of three cases. Cancer 32:917–921, 1973.
24. Wilkinson EJ, Friedrich EG, Hosty TA: Alpha-fetoprotein and endodermal sinus tumor of the ovary. Am J Obstet Gynecol 116:711–714, 1973.
25. Jimerson GK, Woodruff JD: Ovarian extraembryonal teratoma. Am J Obstet Gynecol 127:73–79, 1977.
26. Gershenson DM, Del Junco G, Herson J, Rutledge FN: Endodermal sinus tumor of the ovary: The M.D. Anderson experience. Obstet Gynecol 61:194–202, 1983.
27. Neubacker RD, Breen JL: Embryonal carcinoma of the ovary. Cancer 15:546–556, 1962.
28. Smith JP, Rutledge FN: Advances in chemotherapy for gynecologic cancer. Cancer 36:669–674, 1975.
29. Kurman RJ, Norris HJ: Embryonal carcinoma of the ovary. A clinicopathologic entity distinct from endodermal sinus tumor resembling embryonal carcinoma of the adult testis. Cancer 38:2420–2433, 1976.
30. Kurman RJ, Scardino PT, McIntire KR, Waldmann TA, Javadpour N, Norris HJ: Cellular localization of AFP and hCG in germ cell tumors of the testis and ovary. Scand J Immunol 8 (Supp 8) 127–130, 1978.
31. Thurlbeck WM, Scully RE: Solid teratoma of the ovary – a clinicopathological analysis of 9 cases. Cancer 13:801–811, 1960.
32. Robboy SJ, Scully RE: Ovarian teratoma with glial implants on the peritoneum. Hum Pathol 1:643–653, 1970.
33. Norris HJ, Zirkin HJ, Benson WL: Immature (malignant) teratoma of the ovary. Cancer 37:2359–2372, 1976.
34. Kurman RJ, Norris HJ: Malignant mixed germ cell tumors of the ovary. Obstet Gynecol

48:579–589, 1976.
35. Mostofi FK, Price EB, Jr: Tumors of the male genital system. Atlas of Tumor Pathology, Series 2. Fascicle 8. Washington, D.C., Armed Forces Institute of Pathology, 1973.
36. Pick L: Epithelioma chorionektodermale. Berl Klin Schnschr 41:158–195, 1904.
37. Fox H, Langley FA: Tumours of the Ovary. London, England, William Heinemann Medical Books, 1976.
38. Gerbie MV, Brewer JI, Tamimi H: Primary choriocarcinoma of the ovary. Obstet Gynecol 46:720–723, 1975.
39. Peyron A: Sur la présence des cellules génitales primordiales dans les boutons embryonnaires des embryomes parthénogénètiques chez l'homme. C R Acad Sci (D) (Paris), 206:1680–1683, 1938.
40. Peyron A: Faits nouveaux relatifs a l'origine et à l'histogénèse des embryomes. Bull Cancer (Paris) 28:658–681, 1939.
41. Beck JS, Fulmer HF, Lee ST: Solid malignant ovarian teratoma with 'embryoid bodies' and trophoblastic differentiation. J Pathol 99:67–73, 1969.
42. Nicod J-L: Phénomènes de parthénogénèse dans des tumeurs de l'homme. Bull Soc vaud de Sc Nat 62:495–505, 1945.
43. Simard LC: Tératôme malin de l'ovaire humain, d'origine parthénogénétique vraisemblable. Rev Canad de Biol 9:344–265, 1950.
44. Simard LC: Polyembryonic embryoma of the ovary of parthenogenetic origin. Cancer 10:215–223, 1957.
45. Breen JL, Neubecker RK: Malignant teratoma of the ovary: An analysis of 17 cases. Obstet Gynecol 21:669–681, 1963.
46. Marin-Padilla M: Origin, nature and significance of the 'embryoids' of human teratoma. Virchow's Arch Pathol Anat Physiol 340:105–121, 1965.
47. Takeda A, Ishizuka T, Goto T, *et al.*: Polyembryoma of ovary producing alpha-fetoprotein and HCG: Immunoperoxidase and electron microscopic study. Cancer 49:1878–1889, 1982.
48. Scully RE: Gonadoblastoma. A gonadal tumor related to dysgerminoma (seminoma) and capable of sex hromone production. Cancer 6:445–463, 1953.
49. Andrews J: Streak gonads and the Y chromosome. J Obstet Gynaecol Brit Vommonw 78:448–457, 1971.
50. Teter J: Rare gonadal tumors occurring in intersexes and their classification. Int J Gynaecol Obstet 7:183–187, 1969.
51. Teter J, Bozkowski K: Occurrence of tumors in dysgenetic gonads. Cancer 20:1301–1310, 1967.
52. Talerman A: Gonadoblastoma associated with embryonal carcinoma. Obstet Gynecol 43:138–142, 1974.
53. Manuel M, Katayama KP, Jones HW: The age of occurrence of gonadal tumors in intersex patients with a y chromosome. Am J Obstet Gynecol 124:293–300, 1970.
54. Szokol M, Kondrai G, Papp Z: Gonadal malignancy and 46, XY karyotype in a true hermaphrodite. Obstet Gynecol 49:358–360, 1977.
55. Hart WR, Burkons DM: Germ cell neoplasms arising in gonadoblastomas. Cancer 43:669–678, 1979.
56. Schwartz IS, Cohen CJ, Deligdisch L: Dysgerminoma of the ovary associated with true hermaphroditism. Obstet Gynecol 56:102–106, 1980.
57. Szamborski J, Obrebske T, Starzynska J: Germ cell tumors in monozygous twins with gonadal dysgenesis and 46, XY karyotype Obstet Gynecol 58:120–122, 1981.
58. Talerman A, Jarabak J, Amarose AP: Gonadoblastoma and dysgerminoma in a true hermaphrodite with a 46, XX karyotype. Am J Obstet Gynecol 140:475–477, 1981.
59. Luzzato R, Murray JM, Gallagher HS: Gonadoblastoma associated with malignant teratoma. South Med J 72:624–627, 1979.

60. Schellhas HF, Trujillo JM, Rutledge FN, Cork A: Germ cell tumors associated with XY gonadal dysgenesis. Am J Obstet Gynecol 109:1197–1204, 1971.
61. van Camperhout J, Lord J, Lanthier A, Berard M: The phenotype and gonadal histology in WO/WY mosaic individuals: report of 2 personal cases. J Obstet Gynaecol Br Commonw 76:631–639, 1969.
62. Ascheim S, Zondek B: Die schangerschafts diagnose aus dem Hern durch nachweis des Hypophysernvonderslappen - hormons. Klin Wochenschr 7:1404–1414, 1928.
63. Hobson B, Wide L: The immunological and biological activity of human chorionic gonadotropin in urine. Acta Endocrinol. (Kbh.) 46:632–638, 1964.
64. Vaitukaitis JL, Braunstein GD, Ross GT: A radioimmunoassay which specifically measures human chorionic gonadotropin in the presence of human luteinizing hormone. Am J Obstet Gynecol 113:751–758, 1972.
65. Bergstrand CG, Czar Bb: Demonstration of a new protein fraction in serum from human fetus. Scand J Lab Invest 8:174–178, 1956.
66. Abelev GI, Perova SD, Khramkova NI, Postnikova A, Irlin IS: Production of embryonal alpha-globulin by transplantable mouse hepatomas. Transplant Bull 1:174–180, 1963.
67. Tatarinov YS: Detection of embryospecific alpha-globulin in the blood sera of patients with primary liver tumor. Vopr Med Khim 10:90–91, 1964.
68. Gitlin D, Perricelli A, Gitlin GM: Synthesis of alpha-fetoprotein by liver, Yolk sac, and gastrointestinal tract of the human conceptus. Cancer Res 32:979–982, 1972.
69. Abelev GI: Alpha-fetoprotein as a marker of embryospecific differentiations in normal and tumor tissues. Transplant Rev 20:3–37, 1974.
70. Gershenson DM, Del Junco G, Copeland LJ, Rutledge FN: Rare Germ cell tumors of the ovary: the M.D. Anderson experience (in press)..
71. Ueda G, Hamanaka N, Hayakawa K, *et al.*: Clinical, histochemical, and biochemical studies of an ovarian dysgerminoma with trophoblasts and Leydig cells. Am J Obstet Gynecol 114:748–754, 1972.
72. Case Records of the Massachusetts general Hospital (Case 11–1972). New Engl J Med 286:594–600, 1972.
73. Bahari CM, Lurio M, Schoenfeld A, *et al.*: Ovarian teratoma with peritoneal gliomatosis and elevated serum alpha-fetoprotein. Am J Clin Pathol 73:603–607, 1980.
74. Dizerga G, Acosta A, Kaufman RH, *et al.*: Solid teratoma of the ovary. Gynecol Oncol 3:93–102, 1975.
75. Esterhay PJ, Shamiro HM, Sutherland JC, *et al.*: Serum alpha-fetoprotein concentration and tumor growth disassociation in a patient with ovarian teratocarcinoma. Cancer 31:835–839, 1973.
76. Masopeust J, Kithiei K, Radl J, *et al.*: Occurrence of fetoprotein in patients with neoplasms and non-neoplastic disease. Int J Cancer 3:364–373, 1968.
77. Mawas C, Kohen M, Lemerle J, *et al.*: Serum *a* fetoprotein (fetuin) In children with malignant ovarian or testicular teratomas. Preliminary results. Int J Cancer 4:76–79, 1969.
78. Lippert MC, Javadpour N: Lactic dehydrogenase in the monitoring and prognosis of testicular cancer. Cancer 48:2274–2278, 1981.
79. Liu F, Fritsche HA, Trujillo JM, Samuels ML: Serum lactate dehydrogenase isoenzyme I in patients with advanced testicular cancer. (in press).
80. Zondag HA: Enzyme activity in dysgerminoma and seminoma. A study of Lactic dehydrogenase isoenzymes in malignant diseases (The 1963 Fiske Essay). Rhode Island Med J 47:273–281, 1964.
81. Sheiko MC, Hart WRb: Ovarian germinoma (dysgerminoma) with elevated serum lactic dehydrogenase: case report and review of literature. Cancer 549:994–998, 1982.
82. Awais GM: Dysgerminoma and serum lactic dehydrogenase levels. Obstet Gynecol 62:99–101, 1983.
83. Asadourian LA, Taylor HB: Dysgerminoma. An analysis of 105 cases. Obstet Gynecol

33:370–379, 1969.
84. Mueller CW, Topkins P, Lapp WA: Dysgerminoma of the ovary. An analysis of 427 cases. Am J Obstet Gynecol 60:153–159, 1950.
85. Santesson L: Clinical and pathological survey of ovarian tumours treated at the Radiumhemmet. 1. Dysgerminoma. Acta Radiol (Stockholm) 28:643–668, 1947.
86. De Palo G, Pilotti S, Kenda R, *et al.*: Natural history of dysgerminoma. Am J Obstet Gynecol 143:799–807, 1982.
87. Lucraft HH: A review of thirty-three cases of ovarian dysgerminoma emphasising the role of radiotherapy. Clin Radiol 30:585–589, 1979.
88. Gordon A, Lipton D, Woodruff JD: Dysgerminoma: a review of 158 cases from the Emil Novak ovarian tumor registry. Obstet Gynecol 58:497–504, 1981.
89. Talerman A, Huyzinga WT, Kuipers T: Dysgerminoma clinicopathologic study of 22 cases Obstet Gynecol 41:137–147, 1973.
90. Felmus LB, Pedowitz P: Clinical malignancy of endocrine tumors of the ovary and dysgerminoma. Obstet Gynecol 29:344–350, 1967.
91. Brody S: Clinical aspects of dysgerminoma of the ovary. Acta Radiol (Stockholm) 56:209–230, 1961.
92. Thoeny RH, Dockerty MB, Hunt AB, CHilds DS Jr: Study of ovarian dysgerminoma with emphasis on the role of radiation therapy. Surg Gynecol Obstet 113:692–698, 1961.
93. Malkasian GD, Symmonds RE: Treatment of the unilateral encapsulated ovarian disgerminoma. Am J Obstet Gynecol 90:379–382, 1964.
94. Afridi MA, Vongtama V, Tsukada Y, Piver MS: Dysgerminoma of the ovary: radiation therapy for recurrence and metastases. Am J Obstet Gynecol 126:180–194, 1976.
95. Burkons DM, Hart WR: Ovarian germinomas (dysgerminomas). Obstet Gynecol 51:221–224, 1978.
96. Krepart G, Smith JP, Rutledge F, Delclos L: The treatment for dysgerminoma of the ovary. Cancer 41:986–990, 1978.
97. Boyes DA, Pankratz E, Galliford BW, *et al.*: Experience with dysgerminomas at the cancer control agency of british Columbia. Gynecol Oncol 6:123–129, 1978.
98. Pedowitz P, Felmus LB, Grayzel DM: Dysgerminoma of the ovary. Prognosis and treatment. Am J Obstet Gynecol 70:1284–1297, 1955.
99. Freel JH, Cassir JF, Pierce VK, *et al.*: Dysgerminoma of the ovary. Cancer 43:798–805, 1979.
100. Delclos L, Dembo AJ: Ovaries. In: Fletcher GH (ed), Textbook of Radiotherapy, 3rd edn, Philadelphia, Lea & Febiger, 1980, pp 834–851.
101. Creasman WT, Fetter BF, Hammond CB, Parker RT: Germ cell malignancies of the ovary. Obstet Gynecol 53:226–230, 1979.
102. Cohen SM, Goldsmith MA: Case Report. Prolonged chemotherapeutic remission of metastatic ovarian dysterminoma. Report of a case. Gynec Oncol 5:299–304, 1977.
103. Weinblatt ME, Ortega JA: Treatment of children with dysgerminoma of the ovary. Cancer 49:2608–2611, 1982.
104. Newlands ES, Begent RHJ, Rustin GJS, Bagshawe KD: Potential for cure in metastatic ovarian teratomas and dysgerminomas. Brit J Obstet Gynecol 89:555–560, 1982.
105. Einhorn LH: Chemotherapy of metastatic seminoma. In: Testicular Tumors: Management and treatment, New York, Masson Publishers, 1980, pp 151–167.
106. Simon SD, Srougi M, Goes GM: Treatment of advanced seminoma with vinvlastine (VBL), actinomycin D (AcD), cyclophosphamide (CTX), bleomycin (BLEO) and cis-platinum (CPDD). Proc Am Soc Clin Oncol 2:132, 1983 (C-517).
107. Samuels ML, Logothetis CJ: Follow-up study of sequential weekly pulse-dose cis-platinum for far-advanced seminoma. Proc Am Soc Clin Oncol 2:137, 1983 (C-535).
108. Stanton GF, Bosl GJ, Vugrin D, *et al.*: Treatment of patients (pts) with advanced seminoma with cyclophosphamide, belomycin, actinomycin D, vinblastine, and cisplatin (VAB-

6). Proc Am Soc Clin Oncol 2:141, 1983 (C-551).
109. Dougherty CM, Lund CJ: Solid ovarian tumors complicating pregnancy. Am J Obstet Gynecol 60:261–272, 1950.
110. Creasman WT, Rutledge F, Smith JP: Carcinoma of the ovary associated with pregnancy. Obstet Gynecol 38:111–116, 1971.
111. Novak ER, Lambrou CD, Woodruff JD: Ovarian tumors in pregnancy. Obstet Gynecol 46:401–406, 1975.
112. Karlen JR, Akbari A, Cook WA: Dysgerminoma associated with pregnancy. Obstet Gynecol 53:330–335, 1979.
113. Gershenson DM, Del Junco G, Copeland LJ, Rutledge FN: Mixed germ germ cell tumors of the ovary: the M.D. Anderson experience (in press).
114. Gallion H, Van Nagell JR, Jr, Powell DF *et al.*: Therapy of endodermal sinus tumor of the ovary. Am J Obstet Gynecol 135:447–451, 1979.
115. Jimerson GK, Woodruff JD: Ovarian extraembryonal teratoma II. Endodermal sinus tumor mixed with other germ cell tumors. Am J Obstet Gynecol 127:302–304, 1977.
116. Forney JP, DiSaia PJ, Morrow CP: Endodermal sinus tumor. A report of two sustained remissions treated postoperatively with a combination of actinomycin D, 5-fluorouracil, and cyclophosphamide. Obstet Gynecol 45:186–189, 1975.
117. Karlen JR, Kastelic JE: Endodermal sinus tumor of the ovary: an improving prognosis. Gynecol Oncol 10:206–216, 1980.
118. Wider JA, Marshall JR, Bardin W, *et al.*: Sustained remissions after chemotherapy for primary ovarian cancers containing choriocarcinoma. N Engl J Med 280:1439–1442, 1969.
119. Goldstein DP, Piro AJ: Combination chemotherapy in the treatment of germ cell tumors containing choriocarcinoma in males and females. Surg Gynecol Obstet 134:61–66, 1972.
120. Slayton RE, Hreshchyshun MM, Silverberg SG, *et al.*: Treatment of malignant ovarian germ cell tumors. Response to vincristine, dactinomycin, and cyclophosphamide (preliminary report). Cancer 42:390–398, 1978.
121. Curry SL, Smith JP, Gallagher HS: Malignant teratoma of the ovary: prognostic factors and treatment. Am J obstet Gynecol 131:845–849, 1978.
122. Cangir A, Smith J, van Eys J: Improved prognosis in children with ovarian cancers following modified VAC (vincristine sulfate, dactinomycin, and cyclophosphamide) chemotherapy. Cancer 42:1234–1238, 1978.
123. Samuels ML, Holoye PY, Johnson DE: Bleomycin combination chemotherapy in the management of testicular neoplasia. Cancer 36:318–326, 1975.
124. Einhorn LH, Donohue J: Cis-diamminedichloroplatinum, vinblastine, and bleomycin combination chemotherapy in disseminated testicular cancer. Ann Intern Med 87:293–298, 1977.
125. Julian CG, Barrett JM, Richardson RL, *et al.*: Bleomycin, vinblastine, and cis-platinum in the treatment of advanced endodermal sinus tumor. Obstet Gynecol 56:396–401, 1979.
126. Lokey JL, Baker JJ, Prince NA, *et al.*: Cisplatin, vinblastine, and bleomycin for endodermal sinus tumor of the ovary. Ann Intern Med 94:56–57, 1981.
127. Williams S, Slayton R, Silverberg S, *et al.*: Response of malignant ovarian germ cell tumors to cis-platinum, vinblastine, and bleomycin (PVB). Proc Am Assoc Cancer Res and Am Soc Clin Oncol 22:463, 1981 (C-509).
128. Jacobs AJ, Harris M, Deppe G, *et al.*: Treatment of recurrent germ cell tumors with cisplatin, vinblastine, and bleomycin. Obstet Gynecol 59:129–132, 1982.
129. Wiltshaw E, Stuart-Harris R, Barker GH, *et al.*: Chemotherapy of endodermal sinus tumour (yolk sac tumor) of the ovary: preliminary communication. J R Soc Med 75:888–892, 1982.
130. Carlson RW Sikic BI, Turbow, *et al.*: Cisplatin, vinblastine, and bleomycin (PVB) therapy

for ovarian germ cell cancers. Proc Am Soc Clin Oncol 2:156, 1983 (C-609).
131. Berkson J, Gage RP: Survival curve for cancer patients following treatment. J Am Stat Assoc 47:501Ê515, 1952.
132. Samuels ML, Johnson DE, Holoye PY: Continuous intravenous bleomycin therapy with vinblastine in stage III testicular neoplasia. Cancer Chemother Rep 59:563–570, 1975.
133. DiSaia PH, Saltz A, Kagan AR, Morrow CP: Chemotherapeutic retroconversion of immature teratoma of the ovary. Obstet Gynecol 49:346–350, 1977.
134. Horning SJ, Hoppe RT, Kaplan HS, *et al.*: Female reproductive potential after treatment for hodgkin's disease. N Engl J Med 304:1377–1382, 1981.
135. Warne GL, Fairley KF, Hobbs JB, Martin FIR: Cyclophosphamide-induced ovarian failure. N Engl J Med 289:1159–1162, 1973.
136. Sisis ES, Leventhal BG, Vaitukaitis JL: Effects of childhood leukemia and chemotherapy on puberty and reproductive function in girls. N Engl J Med 294:1143–1146, 1976.
137. Blatt J, Poplack DG, Sherins RJ: Testicular function in boys after chemotherapy for acute lymphoblastic leukemia. N Engl J Med 304:1121–1124, 1981.
138. Stillman RJ, Schinfeld JS, Schiff I, *et al.*: Ovarian failure in long-term survivors of childhood malignancy. Am J Obstet Gynecol 139:62–66, 1981.
139. Forney JP: Pregnancy following removal and chemotherapy of ovarian endodermal sinus tumor. Obstet gynecol 52:360–364, 1978.
140. Holmes GE, Holmes FF: Pregnancy outcome of patients treated for Hodgkin's Disease: a controlled study. Cancer 41:1317–1322, 1978.
141. Javaheri G, Lifschez A, Valle J: Pregnancy following removel of and long-term chemotherapy for ovarian malignant teratoma. Obstet Gynecol 61:85–95, 1983.

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