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Dedication

To the administration and staff of Winthrop-University Hospital, including Directors, Hon. Alfred S. Robbins and George Farrell, Esq.; President and CEO, Martin Delaney; Chief of the Department of Medicine, John Aloia; and many others, for over a decade of generous support and encouragement

Contents

Preface

In our environmentally conscious society, reports of an increase in risk for the development of several cancers, including melanoma, lymphoma, and lung cancer, have excited great controversy. Of these tumors, melanoma has demonstrated the most spectacular increase, generating a considerable body of research. The justification for this volume is found in the need for an up-to-date review of this research.

The first chapter of H.K. Koh et al. addresses the question of etiology, including environmental factors, and the second chapter by Weinstock probes the question of worldwide increased incidence. Regardless of the etiologic factors involved, however, it is clearly critical that we establish clinical criteria and educational strategies to promote early diagnosis for both cutaneous and ocular melanoma. As pointed out by L.S. Albert et al. early attempts at such educational interventions appear to be paying off with reports of a higher percentage of curable melanomas presenting to physicians.

Melanoma provides a unique opportunity to observe the early histopathology of a malignancy and is also somewhat unique in the role that microstaging has played in the prognosis and management of this disease, as demonstrated by A.J. Cochran et al. Because we have established that actinic radiation is a major, although not the only, factor in melanoma etiology, we have the basis upon which this malignancy might be prevented, as shown in the armamentarium of preventive strategies. We are learning more about a variety of membrane and growth factors that may give us insight into mechanisms of metastatic spread, as well as the pattern of that spread as discussed by M.A. Warso et al. Hopefully, this knowledge will also lead, in the not-too-distant future, to interventions that may discourage or prevent tumor metastasis.

Ocular melanoma is the most important primary tumor of the eye, and the contrast between its etiology, epidemiology, and unique pattern of metastatic spread is described by J.A. Sahel et al. The therapy of this tumor is determined by its unique anatomy and natural history. Striking advances have recently been made in our understanding of the cytogenetics and molecular genetics of solid tumors. Melanoma has been one of the primary tumor types of benefit from this increasing knowledge, as reviewed by A.P.

Albino et al. A significant minority of melanomas appear to have a familial history, as reported by M.H. Greene. Our increasing understanding of possible genetic factors that may contribute to this familial distribution and phenotypic marker by which it may be recognized are important advances.

Melanoma is historically one of the first tumors in which serious study of tumor immunology and immunotherapy has been carried out. Immunotherapeutic strategies exploiting both humeral and cellular immune mechanisms, nd utilizing tumor cell extract vaccines, immunocompetent cells, and cytokines, have all played a major role in the study of melanoma as described by W.D.-Y. Quan et al. An introduction to the role of membrane and growth factors in the early genesis and natural history of melanoma is provided in the chapter by D.L. Coppock et al. If such factors are important in initiating and sustaining melanoma, inhibition of their production and secretion may play an important role in future therapeutic strategies. Surgery of melanoma remains today, as it was over 80 years ago, the keystone of therapy of early stage disease. However, the extent of primary surgery, the use of elective regional node dissection, and the criteria for palliative surgery in melanoma are still controversies and are discussed by D.M. Coit et al.

Melanoma remains one of the most chemoresistant malignancies known to date. L. Nathanson et al. describe the current state of the art and also briefly review some new approaches to systemic chemotherapy of melanoma that may give promise for the future. M.J. Dougherty et al. demonstrate that although melanoma has always been considered a radioresistant tumor, a fraction of melanomas, in fact, are radiosensitive. Radiotherapy remains the standard treatment for CNS metastasis, an all-too-common metastatic pattern in melanoma. The radiobiology of this tumor is reviewed, and approaches to be used in the future are suggested.

It is hoped that this book will represent a fairly comprehensive review of both current research and the clinical management of melanoma, arranged in such a way as to be maximally user friendly to both basic scientists and clinicians who are interested in this fascinating disease.

We would like to Joan M. McNicholas for work on the organization and preparation of the manuscript, Jeffrey K. Smith for help and understanding about delays in the final version of the manuscript, the late William McGuire, M.D., whose premature death is a loss to all of us, for encouragement to participate in the Kluwer series on cancer treatment and research, and to my wife Anna and my children Andrew, Aran, and Nicholas for patience with my absences resulting from work on the book.

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1. Etiology of melanoma

Howard K. Koh, Thomas H. Sinks, Alan C. Geller, Donald R. Miller, and Robert A. Lew

1. Introduction

Rising melanoma incidence and mortality rates in the United States and throughout many light-skinned populations in the industrialized world have stimulated interest in the etiology of melanoma [1]. Research has focused on genetic and environmental factors. While the precise etiology of melanoma is unknown, research has centered on the role of sunlight and ultraviolet radiation, the possible exacerbating effects of ozone-depletion, precursor lesions, and possible occupational and chemical exposures [2]. In this chapter we explore these four areas.

2. Sunlight and ultraviolet (UV) radiation

The evidence that some aspect of excess sun exposure and ultraviolet (UV) light contributes to the development of melanoma comes largely from retrospective case-control studies. An abundance of indirect evidence supports this theory. As with many other types of cancer, melanoma lacks a definitive laboratory animal model that supports the sunlight etiology. Similarly, relevant data from in vitro experiments are sparse.

General evidence to suggest a link between sunlight and melanoma comes from latitude studies, racial patterns, the entity of xeroderma pigmentosum, phenotype patterns, migration and 'critical period' studies, and analyses of body site distribution, lentigo maligna melanoma, and melanocytic nevi.

2.1. General evidence

Worldwide melanoma rates tend to increase among Caucasian populations as latitude approaches the equator. Muir et al., reporting in 1987 on worldwide cancer incidence, have described this inverse relationship between incidence and latitude as a quadratic equation [3]. Specifically, incidence and mortality rates in Australia, North America, England and Wales, and Scandinavia follow such a pattern [3–6]. However, not all regions demonstrate consistent

L. Nathanson (ed.), Current Research and Clinical Management of Melanoma.

latitude gradients. Inconsistencies occur, such as the lack of such a latitude gradient in Finland (after controlling for urban-rural residence patterns) and in Western Australia [7]. Factors such as heterogeneous ethnicity, vacation habits, migration patterns, and the mix of age, sex, and socioeconomic status could account for some of these deviations.

Variations in melanoma rates by race and pigmentary features also support a possible role of sunlight in melanoma. Melanoma incidence among Caucausians with light skin greatly exceeds that among blacks, suggesting that darker pigment protects against melanoma; for example, in the United States whites are 8–19 times more likely than blacks to develop melanoma [8]. In Hawaii, Hinds found a similar ratio when comparing whites to Asians [9]. Holman and Armstrong measured skin color with a reflectometer and found a higher risk for those with fairer skin [10]. These observations can suggest either a protective role of melanoma against UV light or possibly that decreased numbers of melanocytic nevi in more pigmented populations reduce the number of potential precursor targets. Compared to whites, blacks and Asians more frequently develop melanoma on sun-protected body sites, such as the palmar and plantar surfaces, and mucous membranes [8]. This suggests that melanoma also has etiologies unrelated to sunlight.

Xeroderma pigmentosum (XP), a rare autosomal recessive disorder, is characterized by defective repair of DNA damage induced by UVB [11]. These patients have more than a 1000-fold risk of developing skin cancer, including melanoma, compared to the general population [12]. Kraemer found reduced unscheduled DNA synthesis in all tissues examined (including melanocytes and nevus cells), indicating damage consistent with that induced by UVB [12].

Case-control studies have identified a cluster of phenotypic traits, such as blue eyes, red or blond hair, light complexion, freckles, and poor tanning ability, associated with an excess risk of melanoma [13]. Genetic factors influence these phenotypic factors, the expression of melanocytic nevi, and the overall susceptibility to melanoma. Because phenotypic traits have varied expression, no single trait, such as hair color, has emerged as the dominant risk factor across many studies. Until specific genes are identified, statistical methods cannot explain this variation. Some attention has focused on red hair as a strong risk factor [14,15]. Persad et al. and Elder noted that pheomelanin, contained in the melanocytes of red hair, reacts to UV in a process associated with mutagenesis [16,17]. Other analyses have focused on poor tanning ability as a risk factor. In their review of case-control studies, Evans et al. found 'inability to tan' a significant risk factor in 15 of 16 studies that asked this question and 'tendency to sunburn' significant in 21 of 24 studies [13]. Beitner et al. found that, compared to controls, the skin of melanoma patients had increased light sensitivity, as measured by minimal erythema dose [18].

A major unresolved issue for those who believe in the sunlight etiology theory is to identify the specific events in childhood that heighten the risk

for the clinical emergence of melanoma later in life (Table 1). Migrant studies from Israel, Australia, and New Zealand, comparing melanoma incidence rates in immigrants to these sunny climates to rates in native-born Caucasians left behind, indicate that risk increases with early arrival and long residency among the immigrants [10,19,20]. Holman and Armstrong have specifically suggested that the critical period for melanoma for immigrants to Australia is arrival before age 10 [10]. Their data show that arrivals after age 15 had only one quarter of the risk for those arrivals before age 10. Weinstock et al. showed that for an American cohort, risk rose inversely with the latitude of residence at ages 15–20 but found no such association for ages over 30 [21].

Other studies have focused on childhood and adolescence, a period in which people tend to receive many episodes of intensive exposure [22]. Lew et al. found excess melanoma risk for those who had more blistering sunburns during adolescence and among those who took more long vacations to sunny areas [23]. Weinstock et al. found excess risk for those reporting two or more blistering sunburns between ages 15 and 20, but not for those over age 30 [21]. However, the results of these New England studies contrasted to negative studies done in Western Canada and Australia [24,25]. Lew et al. showed that with different stratifications of the data, such as male/female and poor tanner/easy tanner, the childhood risk factor changed slightly but remained a measure of too much intense sun exposure [23]. This implies that erythema alone, without pain or blisters, may be a risk factor. However, adults in case-control studies may not recall childhood sun exposures with adequate precision.

The distribution of melanoma by body site also implicates intense sun exposure, as opposed to cumulative exposure. Nonmelanoma skin cancer (NMSC), typically linked to high cumulative lifetime sun exposure, concentrates on the head, neck, and hands of patients. Melanoma distributes more frequently on the backs of men and women, and on the legs of women [26]. Melanoma is comparatively rare at sites typically doubly covered by clothing and infrequently exposed to the sun, such as the breasts and genitalia [27]. Armstrong and others suggest that this pattern links sites such as the back and the legs to too much sun during leisure activities. Temporal trends support this hypothesis [28]. Magnus reported dramatic increases in melanoma incidence on the trunk and limbs, but not the head and neck, over a 20-year period [29]. Stevens corroborated this finding [30]. Elwood et al. found increasing melanoma rates over time for females on the lower limbs and for males on the trunk [5]. Of interest, Stierner et al. demonstrated in humans that UV light causes an increase in melanocytes in shielded, as well as exposed, skin [31]. Thinner clothing and trends in fashion that led to more frequent exposure of women's legs and men's backs, combined with activities such as sunbathing, may all be working together to increase the risk of melanoma [32,33].

Of the four major histopathologic subtypes of melanoma, lentigo maligna

Table 1. 'Critical period studies'

Authors	Location	No. cases/ controls	Critical period	Risk factor	Relative risk (95% confidence intervals)
Paffenberger et al. (1978) [171]	U.S.A.	45/180	'Before college'	Outdoor employment	3.9
Lew et al. (1983)	MA/U.S.A.	111/107	Adolescence	(a) Blistering sunburn (b) 'Poor tanning' (c) Long vacs. in sunny areas	(a) 2.05 (1.18–3.56) (b) 1.93 (1.1–3.3) (c) 2.5 (1.1–5.8)
Holman et al. (1984)	West Australia	511/511	<10 years of age	Immigration to Australia	OR: 1.65 (0.34–7.97)
Elwood et al. (1984)	W. Canada	595/595	Childhood	Severe or frequent sunburn	1.3 (NS)
Holman et al. (1986)	West Australia	507/507	(a) <10 years of age (b) 15–24 years of age	(a + b) Severe sunburn on ≥5 occasions	(a) 1.11 (0.51–2.41) (b) 0.98 (0.53–1.82)
Cristofolini et al. (1987) [172]	N. Italy	103/205	Adolescence	Severe sunburns	0.7 (0.41–1.19) (NS)
Weinstock et al. (1989)	U.S.A.	130/300	15–20 years of age	Latitude of residence	2.2 per 12.6 degrees latitude (1.1–4.2)
Weinstock et al. (1989)	U.S.A.	130/300	15–20 years of age	Blistering sunburn (a) 2 (b) 3–4 (c) ≥5	 (a) 1.8 (1.0–3.4) (b) 1.7 (0.9–3.0) (c) 1.9 (1.1–3.4)

MA = Massachusetts; NS = not significant.

melanoma (LMM) is the one that usually appears on the face or maximally sun-exposed areas of the body. Unlike the other subtypes, LMM is usually associated with dermal solar elastosis, suggesting a possible role of cumulative sun exposure [10,34].

The strongest risk factors for melanoma in case-control studies are the presence of dysplastic nevi (atypical moles), a possible precursor lesion, and higher than average numbers of ordinary melanocytic nevi. Swerdlow et al. reviewed nine case-control studies and found an extreme relative risk of melanoma (RR = 64) in subjects with more than 50 nevi over 2 mm in diameter [35]. With clinically atypical nevi present, even higher risks were obtained. An abundance of nevi would seem to be a genetic propensity, as the studies of familial nevi by Greene and Clark have indicated [36]. However, sunlight appears to increase the expression of nevi. Kopf et al. compared the lateral (sun-exposed) surface of the arm to the medial (sun-protected) surface and found an excess of dysplastic nevi in the sun-exposed area [37]. However, Rampen et al. found no association between sun exposure and the number of nevi [38]. Fitzpatrick and Sober have theorized that either UV induces some melanocytic and dysplastic nevi, which in turn serve as targets for promoters, or more generally, UV acts as a promoter on unstable target lesions [39]. Of interest in this regard, some studies of cultured skin fibroblasts from hereditary melanoma patients show abnormal sensitivity to UV [40].

2.2. *Intermittent exposure hypothesis and further epidemiologic studies*

All these diverse sets of evidence lend credence to the hypothesis that assigns a critical role to intense intermittent sun exposures, such as those obtained during leisure activities, to melanoma causation [28]. According to this intermittent exposure hypothesis, an intense exposure would lead to attendant UV damage to DNA and carcinogenesis. Most studies (though not all) found little association between evidence of chronic UV damage — such as NMSC, solar elastosis, and actinic keratosis — and melanoma [41].

Epidemiologic studies comparing indoor and outdoor workers support the intermittent exposure theory. Professional and technical indoor workers have higher incidence rates of melanoma than outdoor workers, such as farmers and construction workers, while the reverse holds for NMSC [28]. Vagero et al. found higher rates among office workers and lower rates in outdoor workers [42]. In 7 of 10 studies reviewed by Evans et al., incidence rates varied directly with measures of socioeconomic status [13]. Austin and Reynolds found no evidence to support outdoor job sun exposure [43]. Beral and Robinson found an excess of melanoma on the trunk and limbs (but not on the head and neck) of indoor office workers compared to outdoor workers [44]. This evidence weakened the hypothesis put forth by Beral et al. that indoor fluorescent light was a risk factor [45]. Most subsequent studies have failed to confirm the fluorescent light hypothesis [46–48].

We speculate that outdoor workers avoid sunburn exposure or gradually acquire a protective tan to avoid painful sunburns or fatigue that would disrupt their work. However, Dubin et al. still noted an increased risk among outdoor workers who tanned poorly (RR = 3.3), but not among those who tanned easily (RR = 1.5) [49].

Leisure or recreational sun exposure provides a plausible explanation of the risk to indoor workers. Armstrong found that of 10 studies that measured surrogates for leisure sun exposure, five had a positive risk, four showed no risk, and one showed a negative risk [28]. However, the factor of recreational sun exposure, like many other behavioral factors gathered in case-control studies, suffers from imprecision in recall, in definition, and in subjective appraisal [49]. Also, some people with sun-sensitive skin may have worn protective clothing during leisure activities, while others did not, so that the measured amount of time may not accurately reflect the time of risk.

The variable of sunburn history has many of the same drawbacks as a retrospective measure of risk, but such a traumatic event has the advantage of being memorable. Also, as Green et al. observed, sunburn reflects acute high-dose UV exposure delivered to melanocytes [50]. We summarized 13 studies that considered sunburn as a risk factor for melanoma, finding that eight found significant excess risk and five did not. However, many of the nonsignificant studies had risk ratios greater than 1, and several of those had adjusted for other more significant risk factors, such as hair color and eye color. This suggests that phenotype determines sun sensitivity and that behavioral factors such as sunburn have a secondary effect [2]. (Table 2). Dubin et al. found an increased risk for a history of sunburns among poor tanners (RR = 2.9), but no risk among good tanners (RR = 0.8) [51]. Similarly, Elwood et al. found that the risk for history of sunburns became nonsignificant after controlling for the phenotypic trait 'tendency to burn' [24,52]. Obviously, some people who burn easily learn to avoid the sun and therefore have fewer severe sunburns [53].

Critics of the sunlight theory have correctly pointed to inconsistencies. The case-control studies have found strong associations between features that resemble precursor lesions, such as dysplastic nevi and an abundance of moles, and between phenotypic traits that correlate with sun-sensitive skin. In general, the behavioral factors, such as the frequency of short-term intense exposures, have weaker associations with the risk of melanoma. Poor data limit statistical methods that might tease out the behavioral risks from the more dominant risks. Also, as already mentioned, few people who develop melanoma late in life can accurately describe their childhood patterns of sun exposure.

2.3. Laboratory studies

The preceding evidence generally links sun exposure to melanoma, with little indication as to which band of wavelengths carries the higher risk.

Table 2. Sunburn studies*

Author	Location	No. cases/ controls	Sunburn criteria	Relative risk (CI)
Mackie and Aitchison (1982) [173]	Scotland	113/113	Severe & prolonged sunburn during past 5 years	2.8 (1.1–7.4)
Lew et al. (1983)	MA/USA	111/107	Blistering sunburn (adolescence)	2.05 (1.18–3.56)
Elwood et al. (1985a)	W. Canada	595/595	Severe or frequent childhood sunburn	1.3 (0.9–1.8) (NS)
Elwood et al. (1985b)	W. Canada	595/595	'Vacation sunburn score' — very severe	1.4 (NS)
Green et al. (1985)	Queensland, Australia	183/183	Severe sunburn of ≥48 h duration	1.5 (0.7–3.2) (2–5 sunburns) 2.4 (1.0–6.1) (≥6 sunburns)
Sorahan and Grimley (1985)	Birmingham, England	58/333	≥5 painful sunburns 1–4 painful sunburns	4.2 3.0
Elwood et al. (1986)	England	83/83	Sunburn causing pain for ≥2 days	1.5 (0.7–3.5) (NS)
Holman et al. (1986)	W. Australia	507/507	Blistering sunburn	SSM: 1.05 (0.55–2.0) (NS) LMM: 2.78 (0.74–10.44)
Holly et al. (1987) [174]	San Francisco, USA	121/139	'Sunburn Score' of 3+	3.8 (1.4–10.4)
Cristofolini et al. (1987)	N. Italy	103/205	Sunburn — adolescence adult	0.7 (0.41–1.19) (NS) 1.21 (0.71–2.07) (NS)
Osterlind et al. (1988) [175]	Denmark	474/926	Severe sunburn (< age 15)	2.7 (1.6–4.8)
Dubin et al. (1989)	NY/USA	289/527	Severe sunburn with blistering	1.61 (1.04–2.56) Poor tanners: 2.93 (1.34–6.85) Good tanners: 0.79 (0.41–1.50)
Weinstock et al. (1989)	USA	130/300	Blistering sunburn (age 15–20)	1.8 (1.0–3.4) 2 sunburns 1.7 (0.9–3.0) 3–4 sunburns 1.9 (1.1–3.4) ≥5 sunburns

SSM = superficial spreading melanoma; LMM = lentigo maligna melanoma; NS = not significant; MA = Massachusetts; CI = 95% confidence interval; NY = New York.

* Modified, with permission, from Armstrong 1988.

Table 3. Why UVB?*

1. UVB component of solar radiation reaching the Earth's surface has greatest effect on cellular DNA.
2. UVB is a mutagen, causes chromosomal sister chromatid exchange and cell transformation.
3. Blistering sunburns, which are associated with a twofold increased risk for melanoma, are induced by UVB wavelengths.
4. Latitude gradient of UVB reaching the Earth's surface fits with the latitude gradient of melanoma incidence.
5. Melanoma incidence correlates directly with UVB flux measure on Robertson-Berger meters.
6. UVB is immunosuppressive in mouse model.
7. UVB exposure fits with nonmelanoma skin cancer.
8. UVB stimulates hyperplasia of the melanocytic system.

* With permission, from Sober 1987 [176].

Most attention has focused on the role of UV radiation, particularly UVB. Loggie and Eddy, in elaborating the hypothesis that UV holds far more risk for melanoma than other types of radiation, note that ionizing radiation shows no latitude gradient and is not effectively blocked by skin pigment [54]. Also, UVB radiation is most intense near the equator and declines dramatically away from the equator, while UVA and UVC show no such latitude gradient [55,56].

Within the UVR spectrum, UVC is not suspected because it is almost totally absorbed by the ozone layer. UVA and UVB are both tumorigenic in animals. Interestingly, 50% of UVA reaching the skin penetrates to the basal layer, compared to only 5–10% of UVB [57,58]. However, 800–1000 times as much UVA radiation as UVB is required to reach the minimum erythema dose in a given person. The greater percentage of UVA to UVB in the solar spectrum (6.8% vs. 1.7% of solar emissions) contributes up to 15% of the erythema dose at noon, but UVB is more biologically active at the basal layer [58].

Blistering sunburns associated with increased melanoma risk are known to be primarily induced by UVB [23]. Furthermore, in XP patients, the damage to DNA correlated with excessive melanoma rates is the UVB-mediated induction of pyrimidine dimers [12].

Much theoretical evidence links UVB to melanoma (Table 3). UVB is a mutagen and causes DNA strand breaks, as well as crosslinking of protein to DNA in intact human cells. In addition, UVB causes efficient production of pyridimine dimers in human P3 epithelioid cells, with the yield per lethal event declining inversely with increasing wavelength through UVB and UVA [59]. In addition, UV targets include not just DNA but also RNA, proteins, and respiratory electron transport processes [59,60]. Studies in the Amazon molly, *Poecilia formosa*, suggest that UV-induced pyridimine dimers

can lead to tumors [61]. UVB is a normal mitogenic stimulus for melanocytes [62]. In SKH-1 hairless mice, single exposures to UV can induce NMSC [63].

However, both UVB and UVA bands of radiation may play a role in the induction of melanoma [59]. Decades of study of the effects of UVR-induced mutagenesis in *E. coli* have disclosed a variety of patterns of damage and DNA repair mechanisms that depend on the mixture and intensity of UVA and UVB [64].

Hence, UVB could theoretically be involved in initiation, via its mutagenic effect, and in promotion by giving the affected cells a selective growth advantage over normal melanocytes. Some hypothesize that the UV-mediated induction of alteration in melanocytes to cause nevi formation may be one possible manifestation of the initiation step in carcinogenesis [65].

In addition to its potential for initiation and promotion, UVB has been shown to be immunosuppressive in a mouse model by Kripke [66]. Immunosuppression is generally thought to be important in the etiology of human skin cancers; for example, renal transplant patients on immunosuppressive therapy have a higher than expected rate of both melanoma and NMSC in sun-exposed areas [67]. Animal work has shown a systemic, selective immunosuppressive effect of UV induction through specific suppressor cells, which prevent rejection of transplanted tumors as well as development of primary skin cancer [68,69].

While chemicals alone, and combined with UV light, can induce melanoma, until recently UV light alone has not consistently induced melanoma in animal models. Studies of mice exposed to UVB and chemical agents such as DMBA and croton oil [66,70] have resulted in melanocytic tumors that may or may not parallel the etiology of melanoma in humans.

Epstein et al. showed that when hairless mice with 7,12-dimethylbenze(a) anthracene DMBA-induced blue nevi are treated with chronic low-dose UVB radiation, 5 of 18 developed large invasive melanocytic tumors [70]. However, DMBA or UVB may have been acting as a promoter rather than as an initiating agent. Pathak observed the development of melanoma in 38% of a hairless pigmented mouse strain treated with repeated UVR exposure for over 30 weeks. However, melanoma occurred only in those mice that were pretreated with DMBA and who had developed nevi [71]. Kripke subjected 40 mice to 10 UVB exposures over 2 weeks, then applied croton oil; of this group, one mouse developed melanoma at 92 weeks [66]. In support of the role of immunosuppression in melanoma, she also found that transplanted melanoma grew more rapidly in UVB-irradiated hosts than in nonirradiated hosts. Kripke attributed this difference to UVB-induced immunosuppression preventing an adequate host response to the tumor cells [66].

Until recently, animal models for melanoma required a combination of UVB and a chemical agent such as DMBA to induce the disease [70]. Now a new model using UV light alone to induce melanoma in the South American

opossum has emerged [72]. We need further data in this important model before assessing its implications for human melanoma.

Related studies of NMSC implicate UV in the etiology. Brash et al. have recently found, in cells of human squamous cell carcinoma patients, distinctive mutations associated with the p53 tumor suppressor genes that have the 'signature' of UVR mutations [73]. Finally, no in vitro data demonstrate successful transformation of melanocytes by UV light alone.

3. Ozone depletion

The thinning of the ozone layer threatens to increase UVR flux, potentially leading to increased skin cancer and other diseases, as well as deleterious effects on plant and animal life, decreasing crop yields and disrupting the food chain.

The ozone layer lies mostly within the stratosphere, a region roughly 10–30 miles above the surface of the Earth [74]. Chlorofluorocarbons (CFCs) developed in the 1930s and used in refrigerators and air conditioners, have gradually accumulated in the ozone layer, producing 'holes' and general thinning, particularly above the polar regions. The introduction of chemicals such as chlorine, bromine, and nitrogen oxide have destabilized the conversion of oxygen (O_2) and a free oxygen atom (O) into ozone (O_3) and have hastened the breakdown of ozone [2, 56]. While this oversimplified model does not begin to describe the chemistry of the stratosphere, scientists, beginning with Molina and Rowland in 1974, have warned about CFCs depletion of the ozone layer [75].

Since the ozone layer absorbs UVB, complete depletion over densely populated regions might lead to more skin cancer and other adverse effects [2]. The recent dramatic evidence of ozone depletion, the hole in the ozone layer over the Antarctic, brought this issue to the attention of the general public. Farman et al. reported a thinning of 50% in the Antarctic ozone layer [76]. Next, the 1987 Airborne Antarctic Experiment confirmed the presence of elevated levels of chlorine monoxide (the product of CFC reaction with ozone) in the ozone hole. The more global measurements made by the Nimbus 7 satellite with the Total Ozone Mapping Spectrometer charted the depletion over a period of 8 years and found a marked decrease at the poles and an overall drop of 5%, even at the equator [77]. However, Frederick has disputed some of these findings, observing that the drift in the optical properties of the remote Earth orbit sensors could have produced a false trend. However, he accepted the depletion of ozone over the polar regions [78]. Independent observations tend to confirm Frederick's position. The ground-based Dobson spectrophotometer network operating in the Northern Hemisphere over the period from 1957 to 1986 failed to detect a significant thinning of the ozone layer, except for wintertime total ozone.

The unique weather at the South Pole may account for the prominence of

holes in this region of the ozone layer. The potentially modulating nitrogen compounds may freeze during the winter and thus fail to impede the damage from chlorine. Also, bromine compounds seem to have accumulated in the polar stratospheres. Toon and Turco describe how polar stratospheric clouds enhance the destructiveness of chlorine by prolonging its prevalence in a reactive form [79].

Experts gathered by the National Academy of Sciences have studied and reported on CFC destruction of ozone and have spurred government action. In 1978, the United States banned the use of CFCs in aerosol products such as hairsprays and deodorants. In 1987, twenty-three nations, including the United States, agreed to curtail production of CFCs [56].

The intense study of the ozone layer has galvanized efforts to measure the intensity of UV that reaches the surface of the Earth. Surface pollutants, including ozone from sources such as automobile exhaust, technical problems with instruments such as the Robertson-Berger meters, and the resolution of variation in readings over time and place have greatly complicated the evaluation of UVA measurement. The intensity of UV depends on local conditions, such as the weather, industrial pollution, cloud cover, and the window of observation [2]. Bruhl and Crutzen noted that increased ozone near the ground may compensate for losses in the stratosphere [80]. During the summer, low-level ozone tends to absorb more UVB than during the winter because of the shape taken by the field of diffuse UVB radiation when the sun is more than 30 degrees above the horizon. For similar reasons, the shape and intensity of cloud cover affects UVB diffusion. Air pollutants further complicate measurement, as they ebb and flow with the season, the weather, and the time of day. Thus, at any given site, an accurate average measurement requires an extensive set of measurements that capture the potentially wide variations in intensity.

Scotto et al. monitored UVR for over a decade from a network of ground-level monitoring stations in the United States. They found a downward trend in intensity of about 1% per year [55]. These observations were made on photosensitive Robertson-Berger meters installed at various National Weather Service stations. Scotto et al. suggested that meteorological, climactic, and environmental factors may have accounted for these trends [55]. Frederick acknowledges the care taken to calibrate the meters and noted the possibility that the trends resulted from long-term instrument-related drifts [78].

Blumthaler and Ambach attempted to measure UVB intensity in the relatively unpolluted locality of the Jungfraujoch High Mountain Station [81]. They used Robertson-Berger meter measurements gathered from 1981 through 1989, adjusted their estimates for seasonal variation and cloud cover, and found a slight but significant increase in UVB flux of about 1% per year.

Overall, these data indicate that UVB intensity at sea level has not increased dramatically and that so far local conditions such as pollution have

effects of the same magnitude as the thinning ozone layer. These daunting measurement problems led to the comment in a 1991 *Lancet* editorial that attempts in the United Kingdom to 'monitor UV flux at ground level have been pitiful' [82]. However, this does not rule out the possibility that further thinning of the ozone layer could sharply increase UV intensity while local conditions remained comparatively stable. Also, if the threat manifests itself in damage to plants and animals in areas such as the South Pole, which are relatively unprotected by industrial pollution, then the results obtained on the Jungfraujoch may be more indicative [83].

Were large excesses of UVB to reach the surface of the Earth, then assuming the intermittent exposure hypothesis were true, the death rate from melanoma could rise. Longstreth et al. project that a sustained reduction of 10% in the ozone layer, and a corresponding increase in surface UVB would lead to a worldwide excess of about 4500 cases of melanoma per year [56]. Some extra deaths, but an enormous cost, would accumulate from the excess NMSC. Some project the 10% reduction in ozone would add about 300,000 extra cases per year to the millions of cases worldwide [83]. Some of this may be offset by the public's change in their sun-exposure habits to avoid skin cancer.

4. Precursor lesions

4.1. Congenital melanocytic nevi

Melanocytic nevi occur in an estimated 1–2% of newborns. Most are of relatively small size [84–86]. Their risk of evolution into melanoma and their management differ by size of the lesion.

The rare giant congenital melanocytic nevus (GCMN) is a melanocytic nevus at birth of at least 10 cm in diameter; other definitions applied to GCMN include nevi that cannot be removed in a single procedure and those with a diameter exceeding the individuals's palm (if the lesion is on the head or neck), or twice that size (if it is elsewhere on the body) [87,88].

GCMN are extremely rare, with estimates ranging between 0/1058 and 1/1160 in separate U.S. studies to 1/20,500 among South American newborns [84,89,90]. Few prospective studies have studied the evolution of melanoma from GCMN. One study of 151 patients found GCMN progression to invasive melanoma in 4.7%, a risk increased 17-fold as compared to persons without a history of GCMN [88]. (Others speculate that the rate of transformation in this study may be even higher since patients were not enrolled before 8 years of age [91]). About half of the melanoma cases in this study and others were diagnosed in the first 3–5 years of life. Because many studies also note a poorer prognosis for melanoma cases arising in GCMN, many recommend surgical excision of these lesions in early childhood [92–94]. In the afore-

mentioned series of 151 patients, there was little to no morbidity among 50 patients with head and neck giant nevi, although others feel that surgery may be difficult because of the location and size of the lesion [88]. Based on this study, Rhodes et al. estimated a lifetime risk of at least 6.3% for persons with GCMN [94].

Small- to medium-sized congenital melanocytic nevi (SCMN; <10 cm) may sometimes be a precursor of melanoma as well, although the lack of definitive histologic criteria complicates proper analysis [95]. As smaller nevi are far more common than giant nevi, their management has greater implications for surveillance [91,94].

Careful analysis of SCMN transformation to melanoma is hampered by imprecise criteria for the identification of SCMN and poor patient knowledge about the presence of a lesion at birth. While a prospective study would be advisable, greater public awareness of moles would make it unlikely that such a study could ever be done.

Rhodes et al. reported a 21-fold risk of melanoma in patients with a single, small congenital mole (by patient history) compared to a 3- to 10-fold risk by histology [96]. They also found an 'observed' proportion of 3–20% of melanomas developing in small congenital moles [96]. In a separate study, Rhodes et al. examined 234 melanoma specimens and found a dermal nevocellular nevus contiguous with the melanoma in 64 (27.4%) of these specimens. Histologic specimens of congenital nevi were present in 19 of the 64 specimens (none of which were associated with GCMN), or 8.3% of all melanoma [97]. In another study, Kopf found only 4 of 349 melanomas (1.1%) to be associated with congenital melanocytic nevi [98]. From these studies, Rhodes et al. estimated a cumulative incidence risk of 2.6%–4.9% for persons with small SCMN who live up to 60 years of age, an estimated risk 18 times greater than that of the general population [96]. However, in a subsequent report, Rhodes and others cautioned that these projections of histological associations between SCMN and cutaneous melanoma may have overestimated the rate of progression because of the insufficient criteria for reliably distinguishing SCMN as either acquired or congenital [99].

Smaller congenital moles may also be markers of increased risk. In familial melanoma kindreds, persons with dysplastic moles had an 8.5 times greater risk of melanoma if they also had 'congenital-appearing moles' [100–101].

Unlike giant nevi, there is only minimal risk of progression of SCMN to melanoma before 12 years of age that affects recommendations for surgical treatment of small-medium congenital nevi. While some argue that there is insufficient evidence illustrating the increased risk of melanoma in patients with SCMN, still others argue that prophylactic removal of SCMN can be postponed until puberty, the time at which melanoma risk increases and when local (and not general) anesthesia is possible [91]. In one survey, an estimated 50% of physicians recommended excision of the lesions, while an additional 27% recommended observation [102]. Elder argues that early

surgical excision provides immediate assurance and less cost than lifetime follow-up [103]. There have been no trials evaluating changes in mortality or morbidity resulting from excision of congenital nevi.

4.2. Dysplastic nevi or atypical moles

Dysplastic nevi (DN) are melanocytic lesions that appear to constitute both precursors of melanoma, as well as markers of increased melanoma risk [101,104]. Currently, there is little consensus over critical questions concerning dysplastic nevi, including (1) the precise clinical and histological criteria for DN, (2) its prevalence in the general population, and (3) therefore, its significance as a precursor and risk marker for melanoma. Recently, the National Institute of Health (NIH) Consensus Conference recommended using the term *atypical mole* instead of *dysplastic nevi* [105].

In 1976, Clark described distinct clinical and histopathological characteristics in moles of persons from melanoma-prone families [106]. At the same time, Lynch and colleagues described the familial atypical multiple melanoma (FAMMM) syndrome in persons with these atypical moles and familial melanoma [107]. Later, the terms *dysplastic nevi* and *dysplastic nevus syndrome* were established to describe this entity.

Initial studies distinguished dysplastic nevi from common acquired melanocytic nevi by clinical criteria, including their larger size (usually 5 mm or more), irregular borders, greater variety in color, and the likelihood of appearing throughout both childhood and adult life (and not decreasing in numbers with age). The number of lesions ranged from 1 to greater than 100 [108–114].

Over time, investigators found DN in the nonfamilial setting, as well as in persons with a personal or family history of melanoma. Kraemer and colleagues classified persons with DN into five risk categories based on family history of DN. These groups ranged from kindred type A ['sporadic' DN, (with no family history of DN or melanoma)] to type D2 (two or more family members have DN and melanoma) [113]. Based on a prospective study of 401 members of 14 melanoma-prone families, persons with familial dysplastic nevus syndrome (those with dysplastic nevi and two or more first-degree relatives with melanoma) were found to have an estimated relative risk of 148 and a lifetime risk of melanoma that approached 100% [109].

Since such families are rare, the larger question pertains to the prevalence and risk of so-called sporadic (nonfamilial) dysplastic nevi and the progression of melanoma. Investigators found risk estimates for persons with nonfamilial or sporadic DN ranging from 7- to 70-fold, with Kraemer and colleagues estimating that 6% of persons with sporadic DN would develop melanoma during their lifetime [108–114]. However, questions abound as to the relevance of these risk estimates, particularly since varying histopathological criteria have led to vastly divergent estimates of the prevalence of DN. An estimated 20% of patients will have at least one contiguous dysplastic nevus

on histological examination of cutaneous melanoma [94]. Earlier estimates indicated that 1.8%–7% of all whites had at least one DN, but recently Piepkorn found DN in 53% of whites in Utah [115].

Controversies surround not only histological criteria, but also clinical criteria. Epidemiologic studies have differed on the size, frequency, and anatomic distribution of atypical moles. In one study, persons with 12 moles greater than at least 5 mm were found to have an estimated 41-fold increased risk of melanoma [116]. A lesser relative risk of 11 was observed for persons with 10 or more palpable moles in the upper extremities [117].

These controversies relate to proper identification and education of persons with dysplastic nevi or atypical moles, particularly those within the familial melanoma setting. Studies of surveillance of family members in Philadelphia and Europe led to the detection of thinner, early stage melanoma [118,119]. With better criteria and definitions, intensive educational and early detection programs on sun avoidance and early detection with those persons presumed to be at high risk may be promising.

4.3. Lentigo maligna

Lentigo maligna (LM, or Hutchinson's freckle), is a preinvasive lesion, with an unclear risk for progression to invasive melanoma (lentigo maligna melanoma, LMM) [34,120–122]. Most argue that lentigo maligna represents melanoma in situ, with LMM differing from LM only in terms of the presence of abnormal melanocytes below the epidermal basement membrane [123–125]. LMM is most frequently found on the face and is thought to be linked to long-term solar exposure [125–127]. Most case studies find that the age of diagnosis greater than 65 years, about 10–15 years older than the median age of melanoma cases reported to cancer registries [126–129].

LM presents as a large, irregular macule with haphazard pigmentation on sun-exposed skin. Its color is similar to that of a stain on the skin, often tan-brown, with differing shades throughout. Areas of dark brown or black may appear as the lesion grows larger. Regression of the lesion may be indicated by areas of 'lightening.' In later stages, small indurated nodules, which are signs of invasive LMM, may develop [101,126–128,130].

The interval between LM and the onset of LMM is highly variable. While it is commonly accepted that lentigo maligna has a long duration, these studies may be unreliable, as they are generally based on patient recall. Most LMM patients report that the precursor form has been apparent for at least 5–15 years [131].

While some have estimated that a fourth to half of all cases of LM evolve to invasive melanoma, Weinstock and Sober projected that less than 5% of LM progresses to invasive cancer by analyzing data from the NHANES 1 study, SEER, and three hospital-based registries [121,126,128]. They acknowledged that the estimate may be biased, however, because clinical diag-

noses of LM from the NHANES 1 study were never histologically confirmed, providing only a crude estimate of its prevalence [121].

While there appears to be an increase in the diagnosis of melanoma in situ, there is little information on trends in the diagnosis of LM [132]. A study from Queensland, Australia (1977–1979), noted that the rates of tumor with an in-situ component of Hutchinsons Melanotic Freckle (HMF) appeared to be rising rapidly [133].

Metastatic disease and death may result from the development of invasive LMM within LM [126,134]. While LM is generally considered to be slow growing, there have been reports of rapidly evolving cases. These lesions became deeply invasive despite close medical supervision, suggesting that follow-up intervals of 3–6 months for examination of LM patients may be indicated [126,134].

Some authors recommend surgical excision of all lentigo maligna [134]. However, surgical excision is not without its adverse effects, particularly with the morbidity incurred as a result of surgery of large facial lesions among an elderly population.

5. Occupation and chemical exposures

Epidemiologic studies of workers, occupational studies, can provide valuable information by measuring and validating defined population exposure. Also, workers are usually more heavily exposed to carcinogens than persons in other settings, and fewer persons must be followed to observe an effect. Even so, occupational epidemiology is limited by factors involving study design and size, misclassification of exposure and/or disease, confounding, and various sources of bias. In addition, consideration must be given to the reason for the study. Was it the result of an *a priori* hypothesis or are the authors simply reporting their *a posteriori* findings?

Austin and Reynolds have reviewed the literature regarding occupation and melanoma [43]. Surprisingly, there have been relatively few epidemiologic studies specifically designed to examine the association between melanoma and chemical exposures in the workplace setting. Perhaps this is due to the relative rarity of melanoma (until recently) or because there are few chemical exposures that are thought to cause this disease. Regardless, the literature regarding melanoma and the workplace is surprisingly consistent.

We divide our summary of occupation and melanoma according to study design, focusing on some of the more persistent associations. First, we summarize the reports that link the cancer mortality statistics or cancer registry data with occupational and/or industry statements. These reports provide useful clues for follow-up investigations but do not establish a cause and effect association between melanoma and a specific agent. Next, we summarize selected cohort studies of workers that have reported associations with malignant melanoma.

5.1. Crosslinkage of mortality or incidence data with occupation

Perhaps the most consistent findings regarding melanoma and occupation have come from reports utilizing occupational statements available from death certificates or cancer registries. Greater than expected proportion of deaths appear to occur among clerical workers, accountants, office managers, teachers, clergy, and professionals (Table 4). Gallagher et al. have suggested that the increase in deaths from malignant melanoma in administrators, managers, teachers, and accountants may be linked to workers with increased susceptibility to sunburn [135]. Accordingly, the high income and increased leisure time enjoyed by these workers provide ample opportunity for outdoor sun exposures and serious sunburn. Further work must explore the leisure time activities, fluorescent light exposures, and chemical exposures of these workers.

Various occupations that involve chemical exposures have also been identified. A disproportionate number of chemists were diagnosed with melanoma in the Los Angeles tumor registry [136]. Affected chemists were later interviewed and reported more frequent exposures to various organic chemicals, plastics, and ionizing radiation than chemists without melanoma. In a review of British Columbia death certificates from 1950 to 1978, a greater than expected number of deaths from melanoma was found for chemists and chemical engineers [135]. Dubrow and others have described an excess proportion of deaths from melanoma among printers in Rhodes Island (see Table 4) [137–140]. More recently, Hall and Rosenman reported an increased proportional incidence of melanoma among blue collar workers manufacturing rubber and plastic products in New Jersey [141].

Table 4. The crosslinkage of mortality or incidence data with occupation

	Mortality studies					Incidence studies		
Occupation	1	2	3	4	5	6	7	8
Office workers	+	+		+	+	+	+	+
Professionals	+	+				+	+	+
Teachers		+	+	+	+	+	+	+
Clergy			+	+			+	
Paper/printing	+	+	+	+		+	+	
Engineers			+				+	
Armed forces		+	+		+		+	
Carpenters		+						
Chemists/chemical worker					+			
					+			

1, Adelstein (1972), England; 2, Logan (1982), England; 3, Milham (1983), Washington State; 4, Dubrow and Wegman (1984), Massachusetts; 5, Gallagher et al., British Columbia; 6, Williams et al. (1977), Third National Cancer Survey; 7, Committee for the Cancer-Environment, Sweden (1980); 8, Austin and Reynolds (1986), San Francisco Bay area.

5.2. Selected occupational cohort studies

A letter to the editor of the *New England Journal of Medicine* announced an increased risk of melanoma among workers exposed to polychlorinated biphenyls (PCBs) [142]. Bahn's letter described three persons with melanoma among a small group of exposed workers. Chemically, PCBs consist of two phenyl rings with multiple chlorine atoms attached. PCBs are liquid at room temperature, vaporize when heated, and may be transformed into more toxic dibenzofurans and dioxins. PCBs are absorbed through the skin or inhaled as vapor. Their half-life increases with the number of attached chlorine atoms and probably exceeds 5 years. These chemicals were primarily used to prevent heat buildup in electric capacitors and transformers. PCB production in the United States was halted in 1978. Workers exposed to PCBs include those involved in hazardous waste, electrical repair and maintenance, electrical utilities, and the production of electric transformers and capacitors (before 1978) [143,144].

Since the report by Bahn, several cohorts of workers exposed to PCBs have been studied. Brown followed 2567 heavily exposed workers who made electric capacitors but found no excess risk of mortality from melanoma [145]. Bertazzi et al. reported no excess mortality from melanoma among 2100 electric capacitor workers exposed to PCBs in Italy [146]. A small study by Gustavsson et al. was also negative [147]. More recently, Sinks et al. reported eight deaths from malignant melanoma while expecting fewer than two deaths in a cohort of 3588 electric capacitor workers exposed to PCBs [148]. All of the workers who died from melanoma had begun work at least 5 years before their death, and the risk was greatest for those who had worked at the plant for more than 10 years duration. However, the risk of melanoma did not increase with increasing cumulative PCB exposure. Finally, Liss did not observe an excess mortality from melanoma among 1073 Canadian electric transformer workers [149].

The findings regarding PCBs and melanoma are equivocal. While two studies have detected excesses, five others have not. All of these studies used a cohort approach, which requires the follow-up of a large number of exposed workers over many years. Interestingly, there may be a connection between exposures to PCBs and fluorescent lights. All fluorescent light fixtures contain small electrical capacitors that, prior to 1978, incorporated PCBs [150]. When these capacitors burn out, PCBs may be released into the immediate environment. Regardless, not enough information is currently available to draw any conclusions regarding PCBs and melanoma.

Several studies have examined the mortality experience of petrochemical workers and reported excesses of malignant melanoma. Thomas et al. observed almost a twofold excess in skin cancer deaths among unionized petrochemical workers in Texas [151]. However, Waxweiler et al. observed the same number of skin cancer deaths as expected among hourly workers

at a petrochemical plant in Connecticut [152]. Rushton and Alderson studied the workers of eight refineries in Great Britain [153]. They observed 14 melanoma deaths, while expecting fewer than seven. More recently, Marsh et al. reported the mortality experience of 6831 petroleum refinery and chemical plant workers in Texas and observed seven deaths from melanoma [154]. The number of expected deaths was almost identical when the county mortality rates were used for comparison. The studies of petrochemical workers are difficult to interpret since they do not examine the risk of mortality as a function of exposure to any one chemical. The opportunity for exposures in these facilities probably varies widely from person to person and from job to job.

Studies of workers in the electronics and telecommunications industries have reported excesses in malignant melanoma. Investigators have used the Swedish cancer registry data to examine cancer incidence. Vagero and Olin reported a 40% excess number of melanomas (59 total) among Swedish blue collar workers in the electronics industry [155]. Blue collar workers in the Swedish telecommunications industry had an even greater excess risk, with 12 observed cases and fewer than six expected [156]. Another study reported that three electrical engineers, who graduated from the Swedish Institute of Technology in Stockholm during 1930–1979, died of malignant melanoma compared to less than one death expected [157]. Finally, two cases of malignant melanoma were observed, compared to 0.19 expected, in a small group of electrical shop and electrical powerhouse workers at a plant that produces ferrochromium and ferrosilicon [158].

Among the most interesting connections between malignant melanoma and occupation are the reports of increased melanoma risk among workers at the Lawrence Livermore National Laboratory (LLNL) [159]. A California Department of Health Study identified a threefold excess in malignant melanoma incidence (19 cases) among 5100 employees during 1972–1977. The excess was not associated with length of employment but seemed to be greatest among chemists. In a follow-up nested case-control study, 31 LLNL workers with melanoma were compared to 110 LLNL workers who had not developed melanoma [160]. After adjusting for nonoccupational risk factors, excess melanoma risk for melanoma included exposures to radioactive materials (OR = 3.4), volatile photochemicals (OR = 3.6), chemist duties (OR = 9.4), and nuclear testing in the Pacific (OR = 4.5). Although no putative agent has been identified as causing the excess melanoma at the LLNL facility, studies of this work force have continued. Studies of other nuclear workers at the Los Alamos National Laboratory have failed to identify a similar excess risk of melanoma [161,162].

Increased surveillance for skin lesions among the LLNL work force may explain some of the observed excess melanoma risk [163]. The thickness of the melanomas removed from LLNL workers was reviewed [164]. The authors reported that during 1972–1979, LLNL workers with melanoma

may have been identified earlier in the natural history of the disease than other persons in the community with melanoma. Another study has examined whether occupational induction of pigmented nevi had caused the excess of melanoma among the LLNL work force [165]. Several occupational factors were related to the presence of pigmented nevi. To date, the excess of melanoma among the LLNL remains unexplained. Presently, the LLNL work force is routinely screened for melanoma [166].

6. Summary

Although the precise etiology of melanoma remains unknown, much data link sunlight to melanoma. The imperfect evidence associating sun exposure (particularly UVB radiation) with melanoma emerges from human data, obviating problems inherent in extrapolation from animal and other models. However, the mechanism by which sunlight might possibly initiate or promote melanoma remains obscure.

Some clarification should emerge from the potential isolation of genes that carry susceptibility to melanoma in families prone to the disease; such work could serve as a basis to distinguish genetic and environmental influences in melanoma [167]. Continued studies of faulty DNA repair in XP patients may elucidate the steps in mutagenesis and carcinogenesis. Future case-control studies must address the limits on the accuracy of recall and the limits on statistical methods to separate the cluster of phenotypic risk needed in determining biologically effective dose. Animal and in vitro studies must contribute more insight. Further research in the South American opossum models appears promising [72].

Although ozone depletion has been documented, there has been little definitive evidence of subsequent increase of UVB at the Earth's surface. Nevertheless, the threat posed by ozone depletion deserves continued environmental action and public education. The role of precursor lesions, particularly dysplastic nevi/atypical moles, must be clarified with future research.

The distribution of melanoma among various work forces suggests that occupational risk factors may play an important role in the etiology of this disease [168–170]. The consistent reports of excess melanoma among accountants, clerical workers, professional workers, and teachers deserve further study. Furthermore, evidence of excesses in printing and press, petrochemical, and the telecommunications industries require follow-up. Carefully planned studies that account for nonoccupational risk factors are recommended.

Research over the last four decades has brought much information about melanoma etiology. More work is needed to learn the precise cause and ultimately to prevent avoidable mortality from malignant melanoma.

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2. Epidemiology of melanoma

Martin A. Weinstock

1. Incidence

1.1. Race and ethnicity

One of the most striking features of melanoma epidemiology is the dramatic differences in patterns observed among racial groups. For other cancer sites, racial differences in incidence may reflect socioeconomic and exposure pattern differences. However, for melanoma it appears to be the cutaneous pigmentation that is responsible for the patterns observed; the groups with the lightest color skin have the highest incidence and those with darker skins have much lower incidence.

The racial gradients in incidence are clearly evident in the United States, where substantial populations of different racial origins live in close proximity and are covered by the same series of population-based cancer registries. The broadest system of cancer registration in the United States is the Surveillance, Epidemiology, and End Results (SEER) program, which includes nine population-based registries that together cover approximately 10% of the population [1]. For the years 1987–1988 the melanoma incidence among whites was $10.9/10^5$/year, but among blacks was $0.9/10^5$/year [2]. The Los Angeles County registry includes large numbers of Hispanics as well as Anglos and African-Americans. The incidence among Anglos, Hispanics, and African-Americans (per 10^5/year during 1972–1982) were 12.1, 3.3, and 1.1 among men and 10.0, 3.6, and 1.0 among women [3].

Various ethnic groups residing in SEER registry areas have analogous incidence patterns (per 10^5/year during 1978–1981): In New Mexico, the rates were 12.1 and 1.7 among Anglos and Hispanics; in the San Francisco area, 10.0 and 1.0 among whites and blacks, and less than 1.0 among those of Chinese, Japanese, and Filipino ethic stock; and in Hawaii, 23.0 among whites but only 1.7 among Japanese-Hawaiians [4]. Earlier data from Hawaii [5], Texas [6], and South Africa [7], and concurrent and later data from Puerto Rico [8,9], demonstrate similar trends. American Indians also appear to be a low risk population [10]. In Berlin (1980–1986) the rate among the

L. Nathanson (ed.), Current Research and Clinical Management of Melanoma.

Turkish population was 1.3, compared to 7.1 among those of German ethnicity [11].

The melanomas that occur among darker skinned ethnic groups differ in anatomic site and related features from those that occur in whites [12,13]. Melanomas in blacks occur most commonly on the soles, palms, subungual areas, and mucosal surfaces are also proportionately more common than among whites [7,14–18]. The high relative frequency on the soles appears to be due to the relative absence of melanoma elsewhere among blacks; the absolute incidence of plantar melanoma differs little among the races [19,20]. Japanese, Chinese, American Indians, and Puerto Rican groups resemble blacks in the relative predominance of acral melanomas [8,21–23]. Other Hispanic groups have intermediate or mixed patterns [24].

Melanoma risk also exhibits a gradient with socioeconomic status within white populations, although this is of much smaller magnitude than the observed racial gradients. Melanoma incidence is greatest among the upper socioeconomic classes [25,26].

1.2. Geography

The second striking feature of melanoma epidemiology is the geographic distribution of the tumor in relation to solar ultraviolet radiation. Among white populations, there is a clear pattern of highest incidence in the most equatorial latitudes. The highest well-documented incidence rates in the world pertain to the population of Queensland, Australia. Their rates are four times the rates found in the United States, which are, in turn, double the Canadian rate and more than three times the rate found in the United Kingdom [2,27–29].

One must consider, however, the uneven geographic distribution of melanoma susceptibility. A key risk factor is sun sensitivity, as measured by ability to tan, susceptibility to sunburn, and hair and skin color. This factor is closely tied to the ethnic and racial composition of the population, but detailed population-based data regarding sun sensitivity beyond simple racial distribution statisitics are typically unavailable. Nevertheless, the profound regional differences in ethnicity demand that careful consideration be given to these factors in geographic comparisons.

The large white-skinned populations of the world derive from European ethnic stock; within European ethnic groups there is a steep gradient of sun sensitivity from the more resistant Mediterranean groups to the more sensitive Northern European groups. The geographic variation in melanoma incidence is largely an interplay of ethnic composition and latitude, the former an indication of sun sensitivity and the latter an indication of ultraviolet flux, and hence 'intensity' of sun exposure (Table 1).

The key role of latitude, and hence sun exposure, can be distiguished from ethnic factors by comparing similar ethnic groups residing at different latitudes, such as the populations of the United Kingdom and Australia

Table 1. Incidence of melanoma worldwide, 1978–82 (adjusted to the world standard)

Area	Incidence (/10^5/year)	
	Men	Women
Australia/New Zealand		
Australia, Queensland	30.9	28.5
Australia, New South Wales	17.1	16.1
New Zealand		
Maori	3.7	1.3
Non-Maori	15.6	21.4
Polynesian	1.5	4.8
Asia		
China, Shanghai	0.3	0.2
Hong Kong	0.9	0.7
India, Bombay	0.2	0.2
Israel		
All Jews	5.8	7.4
Born Israel	9.0	14.5
Born Eur. Amer.	7.3	9.3
Born Afr. Asia	1.3	1.6
Non-Jews	0.3	0.4
Japan, Miyagi	0.6	0.3
Japan, Osaka	0.2	—
Philippines, Rizai	0.5	0.2
Singapore	1.3	0.4
Indian	0.8	0.4
Chinese		
Americas		
Brazil, Sao Paulo	3.5	4.0
Canada	5.3	6.0
Colombia, Cali	3.3	3.0
Costa Rica	1.8	1.4
US, Bay Area		
Black	0.7	0.7
Chinese	0.3	0.2
White	10.3	9.0
US, Connecticut		
Black	1.1	0.2
White	8.4	7.7
US, Hawaii		
Chinese	0.5	2.2
Filipino	1.1	0.9
Hawaiian	1.2	1.2
Japanese	1.7	1.2
White	22.7	18.8
US, Los Angeles		
Black	1.9	1.1
Chinese	0.6	1.7
Japanese	0.2	1.1
Latino	2.1	2.6
White	12.4	10.9
Other white	12.4	10.9
US, Puerto Rico	1.4	1.0
US, Seattle	8.3	7.4

Table 1. (continued)

Area	Incidence (/10^5/year)	
	Men	Women
Europe		
Denmark	5.9	8.4
France, Bas-Rhin	3.1	4.6
German Democratic Republic	2.9	3.6
Germany, Saarland	3.7	4.2
Hungary, Szabolcs-Szatmar	1.5	2.2
Ireland, Southern	2.7	6.3
Italy, Varese	3.5	3.7
Netherlands, Eindhoven	4.0	5.9
Norway	8.9	10.5
Poland, Cracow City	2.2	3.8
Romania, County Cluj	1.2	1.6
Spain, Navarra	2.2	2.4
Sweden	7.2	8.2
Switzerland, Geneva	8.9	9.6
UK		
England & Wales	2.2	3.8
Scotland	2.8	4.6
Yugoslavia, Slovenia	2.4	2.7

Data are from references 27 and 28.

[30]. Similar gradients have been noted within the United States, Canada, Australia, and Scandinavia [4,28,31–33]. The importance of latitude of residence during childhood in determining later risk of melanoma has been confirmed by several studies of migrants within the United States [3,34], from the United Kingdom to Australia [35], and from Europe to Israel [36].

Under the assumption that the close relation of latitude to peak levels of ultraviolet B radiation is primarily responsible for the observed association of latitude with melanoma incidence, the relation of ultraviolet B flux to melanoma incidence can be quantified. The proportion increase in melanoma incidence per unit increase in ultraviolet B defines the 'biological amplification factor' or BAF. Existing estimates of BAF must be taken with several grains of salt, however, both because of potential confounding factors, such as ethnicity, and because latitude is only a rough measure of individual ultraviolet B exposure. Indeed, there remains uncertainty regarding the relative importance of the different ultraviolet B wavelengths in the etiology of melanoma and regarding the contributions of wavelengths outside the ultraviolet B band. Nevertheless, estimates of the BAF can be made from available data, and these estimates generally suggest that melanoma incidence (and mortality) increases by less than 10% for each 10% increase in ultraviolet B flux [37].

No consistant latitude gradient has been established among non-Caucasian groups. However, an analysis of cancer registry data from India provides some evidence that within this dark-skinned Caucasian population, there may be somewhat higher risk among those living at more equatorial latitudes. Even here, however, the trends were not entirely consistent [38].

Latitude is certainly a key determinant of ultraviolet flux, but sun exposure is substantially more complex than can be measured by latitude alone. Other factors are also important, including altitude, local topography and albido, cloud cover, haze, suspended particulate matter, other tropospheric factors such as air pollution, and stratospheric ozone concentration. Crucial factors beyond ultraviolet flux include behavioral and cultural determinants of timing, duration, and frequency of individual sun exposure and degree of personal protection during those exposures by clothing and chemical sunscreens. Fortunately, these factors are more amenable to public health intervention than latitude of residence.

1.3. Gender and anatomic site

The overall association of melanoma with gender is modest compared to its association with race, latitude, age, and calendar year. In most Caucasian populations of the United States, men are at greater risk than women, although there are exceptions, particularly among Hispanics [2,27,28,39–42]. For most of Europe, Israel, Canada, and New Zealand, the pattern is reversed, and for Australia and Asia mixed patterns are observed [27,28].

More consistent, however, is the distinct pattern by anatomic site within gender groups among Caucasians. Men have more melanomas on the trunk, and particularly the back, and women have more on the legs. Canadian, Danish, and Australian data have been used to assess incidence rates per unit surface area of the body part [43–45]. The face and neck were high incidence areas in both sexes, although the incidence of melanoma on the ears of men was the highest of any site. The leg was a low incidence site among men but a high incidence site among women. The back was a high incidence site for men. Abdomen, buttocks, thigh, and hand were low incidence sites for both genders, and the scalp was a low incidence site for women. The correspondence between incidence and sites of sun exposure is striking but not perfect; for example, the explanation for the discrepancy between the face and the hand is unclear. Basal and squamous cell carcinoma of the skin differ from melanoma in their anatomic site distribution: for both histologic types of cutaneous keratinocytic malignancies, the majority of lesions occur on the face. However, some outstanding similarities also characterize the incidence of each of these three common types of skin cancer, including the male excess on the ears and trunk and the female excess on the legs [46].

The relation between ultraviolet B flux and melanoma incidence was quantified for the United States by Scotto and Fears for constantly exposed

sites (head, neck, and upper extremities) and intermittently exposed sites (trunk and lower extremities). The basis for their calculation were ultraviolet B radiation measurements in areas with established population-based melanoma registration, although the anatomic site of registered cases was often unavailable. However, they did have data from special surveys of other melanoma risk factors, including factors indicative of sun sensitivity. The biological amplification factor (BAF) that they defined from their regression analysis is defined as the relative change in disease risk associated with a 10% relative increase in ultraviolet B flux. In models that considered only age and flux, the BAFs for men and women were 8% and 10% for constantly exposed sites and 6% and 5% for intermittently exposed sites. After controlling for other variables, the BAFs for constantly exposed sites were 5% and 6%, and for intermittently exposed sites, 3% and 4% [47].

1.4. Validity of rates

Several methodologic issues must be considered in comparing rates from various regions within one country, internationally, or over periods of time. First, diagnostic criteria may differ. It is reassuring that a reevaluation of specimens from 1930, 1955, and 1980 revealed no substantial changes in criteria [48]. However, the availability of 'dysplastic nevus' as an alternative diagnosis may affect recent melanoma rates, since some of these lesions conceivably may have been classified as melanoma in the past. Furthermore, some areas may include in situ melanomas in routine statistical reports, whereas most do not. Second, completeness of registration may vary among areas and over time. In the United States in recent years, there has been an increasing tendency towards completely outpatient diagnosis and treatment of melanoma. However, some registries are exclusively dependent on hospitals and death certification for their reports on melanoma, so they miss many cases [49]. Furthermore, even in registries that seek reports from other sources, such as laboratories not affiliated with hospitals, the information available from those sources may be incomplete [50].

Perhaps the most limiting difficulty is the common use of out-of-registration-area pathology laboratories for the diagnosis of melanoma [51]. These laboratories are often not involved in routine reporting of melanoma to any jurisdiction, particularly for cases occurring among out-of-state residents. In addition, a significant number of dermatologists will perform the histologic diagnosis of melanoma by themselves, without the assistance of a pathologist in a reporting laboratory. These issues of completeness of ascertainment appear to be considerably less troublesome in certain Canadian provinces and several European countries with more unified health care systems than the United States. However, quite apart from the completeness of registration of diagnoses and various technical issues, there may be differences in the proportion of existing melanomas that are detected. This may depend on the medical sophistication of the population, availability of health care, training

of health care providers in melanoma recognition, and the penetration of public health campaigns for early detection. All of these factors are dynamic and may have substantial short-term effects on incidence; the long-term impact is less clear.

Finally, the reference population to which age-adjusted rates are standardized may vary between reports. The 1970 U.S. standard million is a common but far from universal reference; others may place somewhat greater or significantly less weight on the incidence among the elderly, and hence produce artifactual differences in age-adjusted rates.

1.5. Secular trends

A fourth major feature of melanoma epidemiology is the dramatic increase in incidence among Caucasian populations (Fig. 1). Particularly noteworthy are the data recorded over many decades by long-term cancer registries in North America and Europe.

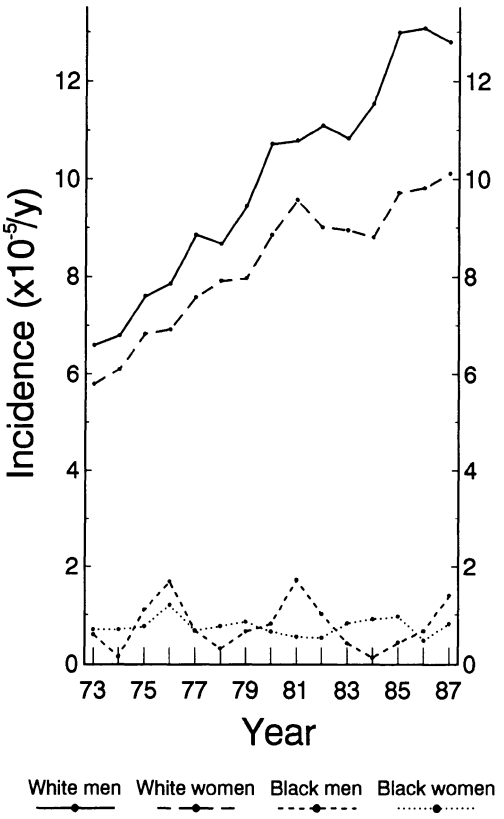

Figure 1. Age-adjusted cutaneous melanoma incidence, United States SEER registries, 1973–1987.

The Connecticut Tumor Registry has tracked melanomas diagnosed in that state since 1935. During the first 5 years of registration, the age-adjusted melanoma incidence was $1.0/10^5$/year; by 1989 this had increased to $12.4/10^5$/year (J. Flannery, unpublished data, 1992). Similarly dramatic increases were noted in the Denmark Cancer Registry, begun in 1943 [52]. Other Nordic coutries that have more than 30 years of data have also reported a doubling of incidence every 10–20 years [32,53–56]. In the United States, registries from New York State (excluding New York City) and from Rochester, Minnesota have noted similar doubling times in incidence during a 35-year period [39,42]. A much larger group of population-based registries have been recording incidence over shorter periods of time. These include registries in Australia and New Zealand [57–61], Europe [11,62–64], Canada [65], Hawaii [5], and the SEER registries in the continental United States [2]. Their data, as well as those from a prepaid health plan in the northwestern United States [66], document similarly dramatic increases in incidence. Indeed, melanoma incidence in the United States appears to be increasing at a faster rate than any other major cancer site [67].

The observed increase in melanoma incidence among whites in so many diverse locations is of such great magnitude that it is difficult to argue that it represents a methodologic artifact, as opposed to a true rise in disease frequency. This conclusion is further supported by the observation that the melanoma mortality rate has also been increasing (see Section 3), although not as rapidly as incidence, and that misclassification of melanoma deaths is not frequent [68]. Changes over time in diagnostic criteria, adequacy of ascertainment, or misclassification would likely produce a period pattern of incidence change, as opposed to the cohort pattern of change observed for melanoma (see Section 1.6). Finally, efforts in both Europe and the United States to look for changes in histopathologic criteria or to adjust for indices of ascertainment have not found evidence for a methodologic artifact sufficient to account for a substantial portion of the observed increases [69,70].

Increases such as those observed over the past 50 years cannot continue indefinately. It may be argued that the lifestyles prevaling in Australasia, Europe, and North America with respect to sun exposure are already maximally melanomagenic, given the existing levels of environmental ultraviolet radiation, and have been so for many years. Since exposures in childhood are particularly important for melanoma formation, under this hypothesis it may be decades before the overall incidence actually declines, but the rate of increase in incidence should soon slow to zero in younger age groups. In light of this hypothesis, it is interesting to note that the melanoma incidence in SEER registries decreased by 9% in 1988, the most recent year for which data are available [2]. The significance of this change is quite unclear, however. The increasing problem of underregistration in the SEER registries in recent years, while still limited in magnitude, may be sufficient to account for the small decrease in incidence observed. It can also be argued that the more common use of dysplastic nevus as a diagnostic alternative for mode-

rately or severely atypical melanocytic lesions may be artifactually lowering the reported incidence of melanoma.

Melanoma incidence has increased at different rates for different anatomic sites of the body. The registry data of several decades duration reveal relatively little increase in incidence on the face, but more explosive increases on the trunk and extremities, particularly the trunk in men and legs in women [32,52,55,56,66,71]. Within the extremities, the increases in the incidence of melanoma on the hands have been quite modest compared to the increases on the remainder of the upper extremity [72]. These have been interpreted to reflect the absence of major changes in sun exposure to the face and hands, compared to increases in exposure of other anatomic sites due to changing styles of dress and activity.

The incidence among blacks in the United States did not appear to change during 1973–1981 [4], nor did the incidence appear to change among non-Caucasians in Hawaii during 1960–1977 [5]. There have been suggestions, however, of an increase in Japan, based primarily on mortality data [20].

1.6. Age, period, and cohort effects

Melanoma is rare in childhood and increases in incidence with age. The association of age with incidence is somewhat irregular when examined cross sectionally, i.e., at a single point in time. Graphs of this relation atypically level off at approximately the sixth or seventh decade before resuming the pattern of increasing incidence with age. One result of this is that melanoma is presently more common than any noncutaneous cancer site in the United States in the 25–29 year age group, but only the ninth most common site overall, excluding basal and squamous cell carcinoma of the skin [2]. If, however, a specific group of people (e.g., those born during a specific 5-year period) are followed through their lifetimes, a more uniform increase with advancing age is apparent. Examination of time trends by looking at the experience of specific groups of individuals as they age has been quite productive for understanding melanoma incidence. When these groups are distinguished by their year of birth, the term for the resulting examination is *cohort analysis*.

To perform a cohort analysis, many years of data are required. Hence, for melanoma, the major cohort analyses have been performed on data from the Connecticut Tumor Registry in the United States and from the Cancer Registries of Scandinavia. These analyses noted the previously mentioned inconsistantcies in cross-sectional age patterns, and in contrast, that the age patterns appeared regular when the experiences of successive birth cohorts were examined. This is interpreted to be evidence for a 'cohort effect,' i.e., that year of birth is more important than year of diagnosis in determining melanoma risk. If the secular increases had been due to changes in an environmental factor that caused melanoma with a short latency period, the age patterns would have appeared more regular when examined cross sec-

tionally than when examined by birth cohort (a 'period effect'), but this was not observed. For melanoma, the observed irregularities of the cross-sectional age curves could therefore be explained by changes in risk of successive birth cohorts [52,71,73].

The cohort effect pertains to each specific anatomic site as well as to melanoma in general. It is least evident for facial melanoma, which has increased the least in incidence. This site also exhibits the most rapid and regular increase in incidence with age when trends are examined at a single point in time (i.e., cross sectionally). The opposite pertains to melanomas of the trunk, and particularly among women, of the extremities. The cohort effect is prominent for these melanomas, which have relatively flat cross-sectional curves of incidence vs. age, and have increased most sharply with incidence over the decades included in the registries.

1.7. Special types and sites

The foregoing discussion pertains to cutaneous melanoma generally. There are also noncutaneous melanomas and subtypes of cutaneous melanomas. Categorization by histologic subtype has not, in general, been found to be productive. There have been suggestions that the lentigo maligna melanomas, which primarily occur on the face, are epidemiologically distinguishable from other types, and are more closely associated with chronic cumulative sun exposure [74]. Under this theory the more common superficial spreading and nodular histologies are more closely associated with intense, intermittent sun exposure. However, it is not yet clear whether the lentigo maligna type differs from other types of facial melanoma. Similarly, acral-lentigenous melanomas certainly differ from other melanomas in their epidemiologic pattern (see Section 1.1), but this may simply be due to their acral location, as opposed to the histologic pattern. Epidemiologic characteristics of less common histologies, such as desmoplastic, borderline, and balloon cell types, are not well established. It should be pointed out, however, that the distinctions among histologic types may sometimes be difficult to reliably define.

Ocular melanomas are discussed in Chapter 7.

Nonocular noncutaneous malignant melanomas (NNMM) occur primarily at mucosal and genital sites, and are quite rare. In the United States, the incidence was less than $0.2/10^5$/year for the years 1969–1971 and 1973–1987 [75,76]. There was relatively little difference between whites and blacks in incidence compared to the cutaneous or ocular sites: The relative risk for whites (vs. blacks) was 1.7 (95% confidence interval 1.1–2.7) compared to 11.7 (95% confidence interval 10.1–13.5) for cutaneous melanoma and 14.3 (95% confidence interval 8.0–26.1) for ocular melanoma in the same registries during the same period. Among whites, there was a strong positive correlation between latitude of residence and incidence, i.e., incidence was higher in more northerly latitudes, in striking contrast to cutaneous melanoma, which

demonstrates the opposite trend, and ocular melanoma, which is not associated with latitude. No overall increase or decrease in incidence was noted during the 15-year period of observation [76].

The pronounced differences in incidence patterns between NNMM and cutaneous melanoma suggest similarly pronounced differences in etiology, despite sharing an identical cell of origin. This argues against the theory that a sun-induced 'solar circulating factor' accounts for much of melanoma incidence, and in particular accounts for the relative lack of melanomas on the face, compared to cutaneous basal and squamous cell carcinomas [77]. Studies have not found a site-specific link between sun exposure and melanoma location on the skin [78,79]. However, the evidence does suggest a site-specific link between sun exposure and melanoma when site is broadly defined as nongenital skin vs. genitalia or mucous membranes.

The reason for the increase in NNMM incidence with increasing latitude is obscure. The observed association may even be interpreted to suggest that sun exposure induces a systemic 'melanoma inhibitory factor' that actually decreases melanoma risk, but that is overwhelmed by the direct carcinogenic effects of ultraviolet radiation in sun-exposed sites [80].

2. Case fatality

Most victims of melanoma become long-term survivors. Hence melanoma mortality rates will not necessarily reflect incidence trends, and many healthy individuals in the population are melanoma survivors. The proportion of the United States Caucasian population that had been diagnosed with melanoma was estimated at approximately 1 in 700 and is increasing rapidly [81–83].

2.1. Secular trends

The case fatality rates have been steadily declining for melanoma over the past several decades among both white men and white women. Data regarding other racial groups is less plentiful. It is clear, however, that black melanoma patients in the United States have a less favorable prognosis than white patients, and men a less favorable prognosis than women (Table 2).

2.2. Prognostic factors

The most important predictor of survivial is the extent of disease at the time of diagnosis. Among patients with localized disease, long-term survival rates are 75% to over 80%, whereas only 15–20% of patients with regional spread of melanoma are long-term survivors, and long-term survival is rare once the melanoma has metastasized to distant sites. (The median survival among patients with distant metastases is approximately 6 months.) The size of the primary lesion, measured by the Breslow thickness (the distance from

Table 2. Five year relative survival rates by year of diagnosis and stage of disease, United States

	% of total	1981–87	1970–73	1960–63
Men				
Overall	100	77	62	51
Localized	78	88		
Regional	9	47		
Distant	5	11		
Women				
Overall	100	87	75	68
Localized	83	93		
Regional	7	55		
Distant	3	20		
Black men and women				
Overall	100	70		
Localized	53			
Regional	13			
Distant	18			

From reference 2.

the granular layer of the epidermis to the deepest tumor cell) is the key determinant of prognosis among those with localized melanoma: Long-term survival is less than 50% if the Breslow thickness is greater than 4 mm, but greater than 90% if the primary lesion is less than $\frac{3}{4}$ mm.

The other factors that affect prognosis depend on the extent of disease at the time of diagnosis. For metastatic disease, the location and extent of metastases are important, but regrettably therapeutic intervention has had little impact on survival. For localized disease, both patient and tumor characteristics have prognostic significance. Older patients have a worse prognosis, although this appears to be primarily due to the association of advanced age with thicker primary tumors. Gender is a second patient characteristic that predicts survival: Men have a less favorable prognosis. However, men also tend to have thicker primary tumors, tumors located in relatively poor prognosis anatomic sites, and more ulcerated tumors. After taking these factors into account, gender appears not to play a major role in determining survival. Anatomic site is an important and independent prognostic factor: primary melanomas located on the head and trunk are associated with poorer survival than those on the extremities, although acrally located melanomas (on the palm, sole, or nail, in particular) are an exception to this observation and appear to have a particularly poor prognosis. Within these broad anatomic areas there are likely additional anatomic distinctions with prognostic importance, such as scalp (poor) vs. face and 'BANS' vs. nonBANS, although these have not been as well established. Additional histologic factors that affect prognosis include ulceration and the mitotic rate

of the primary tumor. Further details of staging and microstaging of melanoma for prognostic purposes are given in Chapter 6.

2.3. Sources of time trends

Insight into the prognostic factors that may account for the trend towards improved survival in recent years requires understanding of trends in the major prognostic factors. However, this degree of detail is typically not available from population-based cancer registration.

One research group has been gathering detailed prognostic information since 1955 on a large group of melanoma patients in the United States at the University of Alabama (UA) and in Australia at the Sydney Melanoma Unit (SMU) [84]. They have noted a marked increase in the proportion of patients presenting with localized disease from 82% and 86% in Australia and the United States in the 1950s to 94% and 92% in the 1980s. Furthermore, among invasive but localized melanomas, the 5-year survival rates increased from 78% and 68% before 1970 to 86% and 82% in the 1980s. Similar trends have been documented elsewhere (Table 2). There were minor changes in age and gender distribution, and a shift in anatomic site from the face to the trunk and extremities in the UA/SMU data, as well as in population-based registries generally. However, the most striking changes were in the histologic characteristics of the melanomas at the time of diagnosis. At both locations, there was a dramatic reduction in both Breslow thickness and the frequency of ulceration (Table 3).

The observation that melanomas are decreasing in thickness at diagnosis has been confirmed in other less extensive data sets and has several possible explanations. The most likely is that melanoma is simply being diagnosed earlier in its natural history because of increased awareness of the clinical signs of early melanoma among the populations studied and their physicians. Other factors that may play a role include a possible change in the biological nature of the disease and a change in diagnostic criteria leading to inclusion of some benign lesions among the melanomas in recent decades. The latter would also lead to an artifactual increase in incidence and was discussed in Section 1.

3. Mortality

3.1. Sources of data

Our knowledge of melanoma mortality rates derives primarily from routine universal death certification. Mortality measures are free of many of the potential problems previously discussed with incidence measures, but nevertheless are imperfect. The validity of the death certificate diagnoses has been studied and has been found to be high [68]. However, the mortality

Table 3. Changes in melanoma characteristics between 1955–60 and 1986–89

	University of Alabama		Sydney Melanoma Unit	
	1955–60	1986–89	1955–60	1986–89
% $<\frac{3}{4}$ mm thick	8%	42%	8%	45%
% >4 mm thick	46%	15%	25%	7%
Median thickness	3.3 mm	1.0 mm	2.5 mm	0.8 mm
% ulcerated	43%	23%	44%	17%

From reference 81.

statistics used cover much greater time periods and geographic areas than the existing validity studies. It also must be kept in mind that mortality trends may reflect either trends in incidence, case fatality, or both.

3.2. Race, latitude, gender, and socioeconomic status

The pattern of melanoma mortality can be predicted on the basis of the foregoing discussion of incidence and case fatality. With respect to race, incidence among Caucasians so exceeds incidence among other races that the mortality also must be higher, despite a substantially lower case fatality rate. For the years 1987–1988 in the United States, the overall mortality was $2.4/10^5$/year among whites but just $0.4/10^5$/year among blacks [2]. Among Caucasians of similar ethnicity, melanoma mortality is strongly associated with latitude; mortality is greatest in more equatorial regions [85]. Among whites in the United States, men die from melanoma at twice the rate of women ($3.4/10^5$/year vs. $1.7/10^5$/year in 1987–1988), but there is no substantial gender difference among blacks [2]. Among Caucasians, the mortality among professional and administrative groups is greater than among those with blue-collar occupations [86].

3.3. Time trends (age, period, and cohort)

The preceding discussion has documented the increasing trend of melanoma incidence in Caucasian populations and the improved survival of those diagnosed with melanoma. Of these trends, the increase in incidence has been the more pronounced over the past decades, and hence one can predict that the observed mortality rates will have increased. Indeed, this has been observed over many decades in the United States, Canada, Sweden, United Kingdom, Australia, and New Zealand. Japanese melanoma mortality rates are also reported to be rising rapidly, although the details of this trend have not been elucidated [13,20]. Increases of approximately 2–3% per year are typical of Caucasian populations. These studies have further observed that the changes over time have followed a cohort pattern, i.e., that risk of death

was more closely associated with year of birth than with year of death; one would expect this on the basis of the much greater magnitude of change in incidence than in case fatality [87–91].

Additional observations from vital records in Australia and New Zealand have noted that the native-born Caucasians have higher death rates from melanoma than those born in Europe [92,93]. This finding also parallels observations regarding incidence rates and is assumed to be a consequence of the key role of childhood sun exposure in determining the risk of melanoma during the adult years.

Two recent analyses of melanoma mortality in the United States during 1950 through 1984 have suggested that the trend of ever increasing mortality rates is ending [94,95]. No actual reversal of the overall increase in age-adjusted mortality was described; indeed, an overall increase from 1.0 to $3.1/10^5$/year was noted for white men and of 0.8 to $1.6/10^5$/year for white women, and more recent data confirm the continuing increase in mortality [96]. However, the rates of increase were lower for both men and women during 1973–1984 than during 1950–1972. Furthermore, when mortality was examined by birth cohort, mortality appears to have peaked among men in the group born in the 1950s and among women in the group born in the 1930s. Based on this observation and the assumption that all other factors do not change, it was projected that actual melanoma mortality rates will peak between the years 2010 and 2020, although the actual number of deaths per year will continue to increase (to approximately double the present number) until about 2030 because of the aging of the population [94].

Figure 2 extends the above observations with an additional 4 years of mortality data. Melanoma mortality increased 66% among men and 23% among women from 1969 to 1988. Among blacks the rates are much lower (one-ninth the white rate among men and one-sixth among women) and statistically less stable because of the small number of deaths. No sustained increase or decrease is discernable among blacks over the 20-year period.

The complex pattern of age-specific mortality among white men is shown in Figure 3. A steady increase in mortality has occurred in all strata over 45 years of age, but actual declines in mortality are observed in more recent years within the younger age groups. The mortality rate among those 15–29 years of age was 11% lower in 1985–1988 than in 1981–1984; among those 30–45 years of age, the decline was 9% (Table 3). Among white women, mortality has also declined in recent years among the younger age groups (Fig. 4).

One of the tragedies of the current melanoma epidemic is that young adults are so commonly affected; indeed, melanoma is more common than any noncutaneous malignancy among Americans 25–29 years of age [2]. Hence we examined the years of potential life lost (YPLL) to melanoma (Fig. 5). Two methods were chosen among the several available to calculate years of potential life lost (YPLL) to melanoma [97]. The CDC method

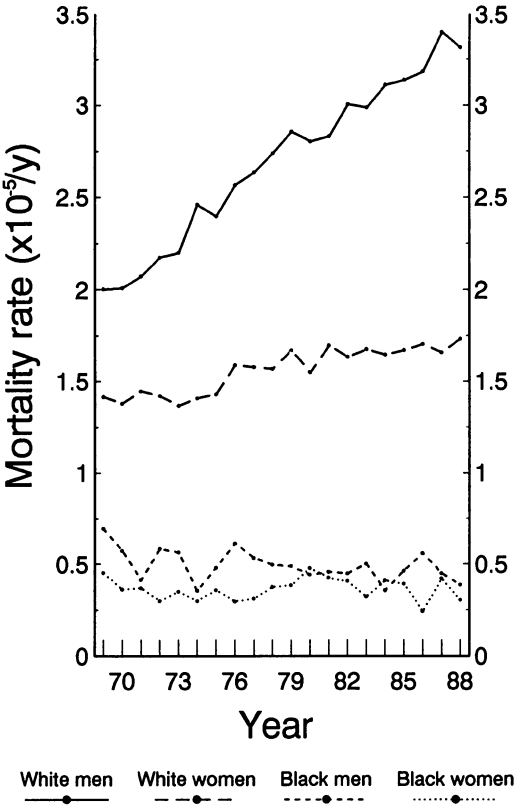

Figure 2. Age-adjusted cutaneous melanoma mortality, United States, 1969–1988.

determines YPLL for a particular death as the difference between the age 65 and the age at death [98]. Deaths after the age of 65 are not considered. The NCI method determines the YPLL for a particular death as the life expectancy of a person of the same race and gender as the deceased at the age of death of the deceased [99]. The present analysis uses the 1979 life table for these calculations [100]. For both methods, the YPLL for all deaths are simply added to determine the YPLL attributable to a particular cause. The CDC method puts greater weight on early deaths than the NCI method, which more closely tracks the overall mortality rate.

Using the CDC computation, a decline in YPLL is evident in the most recent 3–4 years among white men, and among white women YPLL has stabilized or declined slightly. However, no such declines are evident when the NCI computation is used for YPLL.

To better understand the mortality trends, these trends may be compared to the trends in melanoma incidence recorded by the National Cancer

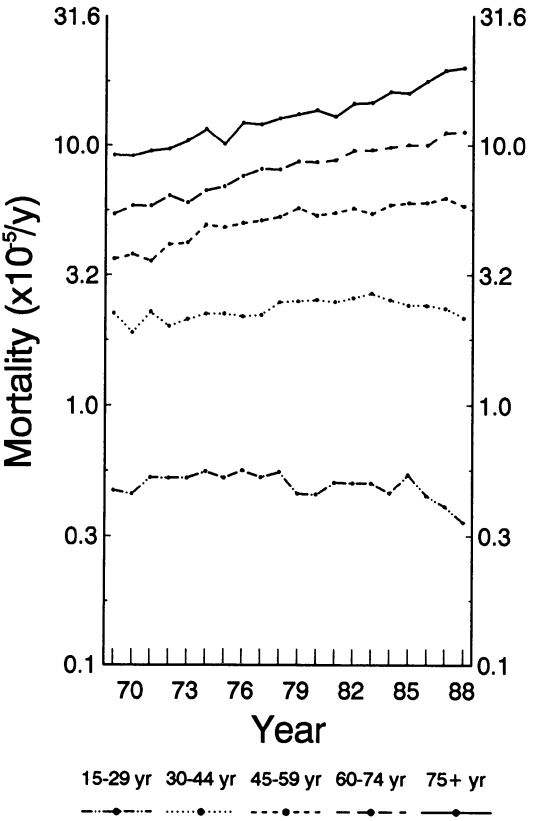

Figure 3. Age-specific cutaneous melanoma mortality, United States, white men, 1969–1988.

Institute Surveillance, Epidemiology, and End Results program (SEER) registries during the period 1973–1987. The age-adjusted incidence rates for white men and white women during this period were steadily increasing, with minor year-to-year fluctuations (Fig. 1). When age-specific incidence rates are examined, no decline is evident for recent years (Table 4).

The interpretation of these mortality trends is complicated by the previously discussed uncertainties in the meaning of the incidence data. Modest changes in incidence could well reflect methodologic factors rather than true trends in disease incidence. In particular, the apparent decline in incidence among white men in 1983 and among white women in 1982 through 1984 may have been due to under-reporting of cases diagnosed in certain outpatient settings; subsequent implementation of more intensive case-finding procedures for individuals diagnosed in the outpatient setting minimized this problem, and the reported incidence was subsequently greater than prior to the temporary decline [50,99]. It is nevertheless plausible that as more

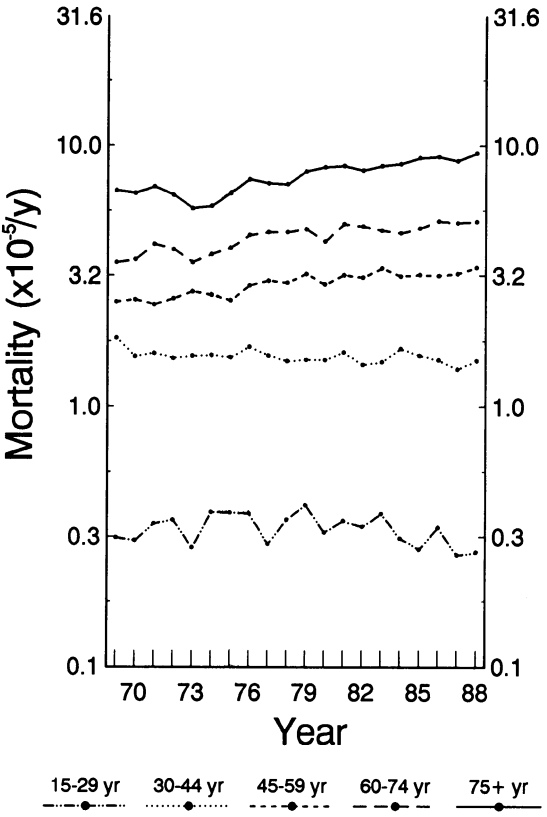

Figure 4. Age-specific cutaneous melanoma mortality, United States, white women, 1969–1988.

procedures are performed in the outpatient setting, under-reporting of outpatient diagnoses may be an increasing problem, even among the SEER registries, particularly among cases of registry area patients who are diagnosed outside the registry areas. If so, the recent increase in melanoma incidence may be underestimated by the reported rate.

Classically, birth cohort patterns in disease frequency are explained by the exposures received and lifestyle patterns determined during childhood or early adulthood. These factors may play an important role in the apparent cohort pattern to the observed reversal of melanoma mortality rates. However, in the face of an apparent continuation of the rise in melanoma incidence, other explanations must be considered.

Younger melanoma patients may be realizing a more substantial decline in case fatality than older individuals. Early detection may be easier in young adults than among the elderly for several reasons. The legs, and particularly the torso, of younger adults may be visible to friends and relatives more frequently. Younger people may be more concerned about

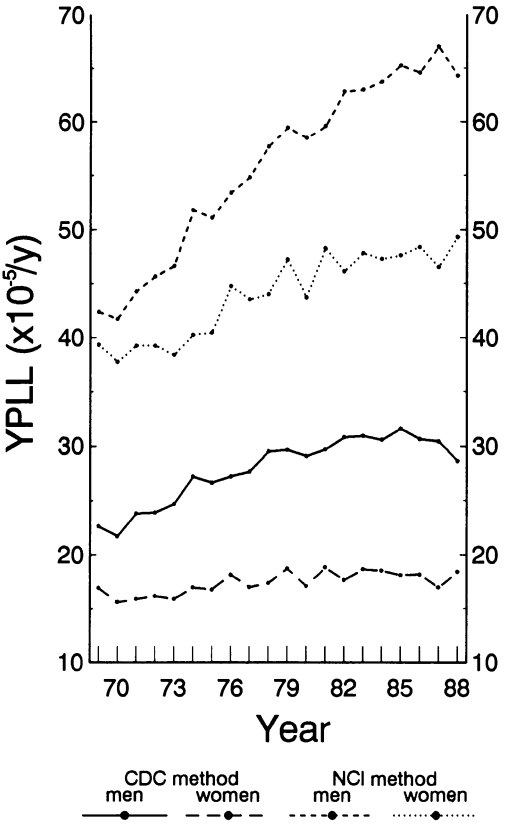

Figure 5. Years of potential life lost (YPLL) to melanoma by sex, United States whites, 1969–1988.

the appearance of their nonfacial skin, where most melanomas arise. The elderly typically have a much larger number of benign skin lesions, such as seborrheic keratoses, that may have many of the warning signs of melanoma (such as asymmetry, multiple colors, and large diameter), which may inhibit recognition of dangerous but similar-appearing lesions or changing lesions. Finally, the elderly are more likely to develop nodular melanoma, which is typically more difficult to recognize at an early stage than the more common superficial spreading type.

A recent report of mortality trends in Sweden also suggests a decline in mortality in recent cohorts. Those data reveal a peak in rates among men born in the 1930s and among women born in the 1940s. However, there was evidence in the data for a 'period' (as opposed to 'cohort') effect among women but not men. Although the overall age-adjusted rates continued to climb in Sweden, among women the 1983–1987 rate was 1% less than the 1978–1982 rate [91].

Table 4. Age-specific melanoma mortality, United States, 1969–1988

	0–14		15–29			30–44			45–59			60–74			75+		
Year	Rate	N	Rate	% Chg	N	Rate	% Chg	N	Rate	% Chg	N	Rate	% Chg	N	Rate	% Chg	N
White men																	
1969–72	0.01	11	0.50		436	2.11		1260	3.79		2206	5.86		2040	9.34		1016
1973–76	0.01	10	0.54	9	538	2.20	4	1394	4.73	25	2803	5.79	16	2530	11.05	18	1276
1977–80	0.01	7	0.50	−8	524	2.43	10	1738	5.35	13	3151	8.33	23	3351	12.89	17	1613
1981–84	0.01	6	0.49	−1	519	2.57	5	2102	5.60	5	3241	9.39	13	4066	14.53	13	1996
1985–88	0.02	13	0.44	−11	450	2.32	−9	2174	5.99	7	3441	10.59	13	4840	18.25	26	2745
White women																	
1969–72	0.01	5	0.34		295	1.63		1003	2.52		1577	3.83		1639	6.64		1166
1973–76	0.00	2	0.36	9	355	1.60	−2	1031	2.71	7	1726	3.98	4	1839	6.39	−4	1293
1977–80	0.01	6	0.35	−4	362	1.52	−5	1100	3.02	12	1912	4.56	15	2284	7.60	19	1726
1981–84	0.01	7	0.35	−0	361	1.55	2	1279	3.19	6	1967	4.78	5	2550	8.29	9	2110
1985–88	0.00	0	0.29	−17	291	1.49	−4	1391	3.23	1	1957	5.01	5	2794	9.02	9	2539
Black men																	
1969–72	0.01	1	0.03		3	0.34		23	0.85		48	2.08		68	3.76		32
1973–76	0.01	2	0.07		9	0.27		19	0.69		40	2.13		76	2.67		26
1977–80	0.01	2	0.02		3	0.12		10	0.71		42	1.83		69	3.95		43
1981–84	0.01	1	0.04		7	0.16		15	0.64		39	1.64		66	3.23		39
1985–88	0.00	0	0.04		7	0.16		18	0.57		37	1.73		75	3.93		52
Black women																	
1969–72	0.00	0	0.03		4	0.10		8	0.64		42	1.66		67	1.70		21
1973–76	0.02	3	0.05		8	0.09		8	0.36		25	1.37		62	2.44		37
1977–80	0.01	1	0.02		3	0.20		19	0.51		37	1.60		79	2.58		46
1981–84	0.02	3	0.04		6	0.13		15	0.52		39	1.43		76	3.35		69
1985–88	0.00	0	0.02		3	0.15		19	0.37		29	1.41		81	2.82		66

Table 5. Age-specific melanoma incidence, SEER registries, 1973–1987

	0–14		15–29			30–44			45–59			60–74			75+		
Year	Rate	N	Rate	% Chg	N	Rate	% Chg	N	Rate	% Chg	N	Rate	% Chg	N	Rate	% Chg	N
White men																	
1973–76	0.08	7	2.89		251	8.54		487	14.83		781	17.36		527	22.73		220
1977–80	0.06	5	3.25	12	318	10.91	28	750	18.66	26	1024	24.98	44	866	30.92	36	334
1981–84	0.08	6	2.86	−12	279	11.50	5	897	22.58	21	1188	32.03	28	1192	37.32	21	435
985–87	0.10	6	3.39	18	241	13.20	15	862	24.84	10	950	38.85	21	1132	49.40	32	462
White women																	
1973–76	0.14	11	3.84		328	9.43		541	12.24		678	11.99		445	17.84		307
1977–80	0.15	12	5.25	37	502	12.23	30	840	15.30	25	881	15.84	32	672	16.97	−5	341
1981–84	0.13	10	4.60	−12	434	14.19	16	1099	18.24	19	1000	17.84	13	805	21.62	27	481
1985–87	0.20	11	5.70	24	389	14.67	3	944	19.38	6	764	20.18	13	710	23.51	9	424
Black men																	
1973–76	0.09	1	0.00		0	0.74		4	1.59		7	2.62		6	5.97		3
1977–80	0.00	0	0.08		1	0.14		1	0.81		4	3.21		9	3.02		2
1981–84	0.00	0	0.08		1	0.24		2	0.81		4	3.54		11	6.45		5
1985–87	0.00	0	0.10		1	0.14		1	0.81		3	2.75		7	9.07		6
Black women																	
1973–76	0.00	0	0.19		2	0.32		2	2.01		10	1.84		5	6.19		5
1977–80	0.00	0	0.08		1	0.37		3	1.23		7	1.70		6	7.13		8
1981–84	0.08	1	0.00		0	0.31		3	1.38		8	2.54		10	4.45		6
1985–87	0.00	0	0.19		2	0.24		2	0.68		3	3.10		10	6.86		8

The experience of Scotland has also been studied and is particularly noteworthy. They also are an area of increasing incidence and with a higher proportion of thick, poor prognosis tumors among the elderly [64,101]. In 1985, a major public education campaign was launched to encourage early recognition of melanoma among the population. The campaign included education of primary care phycisians and prompt referral of suspicious lesions to specialists in the medical care system [102]. At the time of the campaign, there was a sharp increase in the number of patients referred for evaluation of possible melanomas and in the number of melanomas diagnosed. There also was a pronounced decrease in the proportion of thick tumors among those diagnosed. Among women who were over-represented among those referred for evaluation of potential melanomas, the incidence of thick melanomas began to decrease in 1988, although no analogous decrease was noted in men. Most encouraging was the observed decrease in overall melanoma mortality rate among women (but not men) after 1988, a distinct departure from the historical trend of increasing mortality among both gender groups [103]. These fascinating data suggest that the discrete (in time) public health intervention is responsible for the observed trends in both incidence and mortality.

4. The future

It has been observed that prediction is difficult, especially about the future. This applies to melanoma incidence, case fatality, and mortality, although much may be gained by understanding the key parameters that will be determinative.

4.1. Ozone depletion

The stratospheric ozone prevents much of the solar ultraviolet B radiation from reaching the surface of the Earth. This vital component of our atmosphere is being severely depleted by the release of man-made chemicals, such as chloroflorocarbons, at the Earth's surface. It takes many years after release for these compounds to migrate, react, and destroy ozone in the stratosphere, so it can be confidently predicted that even with rapid elimination of the release of these compounds, the ozone layer will continue to weaken for decades.

The future trends in ultraviolet B flux at the surface of the Earth more complex, however. First, the predicted effect of ozone depletion varies with wavelength of ultraviolet B light: According to a recent calculation, a 10% decrease in ozone is projected to result in a 50% increase in flux at the 295 nm wavelength, but only a 10% increase at the 315 nm wavelength [37]. Cloud cover is a key variable that may be affected by global environmental change and have a pronounced effect on ultraviolet B flux [104]. Other factors include air pollutants, such as suspended particulates, which ironically

may protect against skin cancers by decreasing the transmission of ultraviolet rays. Hence, many areas in the United States have not seen a net increase in ultraviolet B flux, despite ozone layer depletion of approximately 5% over the past decade [105; R. Stolarsky, Goddard Flight Center, unpublished data, 1992].

4.2. Individual exposure

The major forces driving melanoma incidence over the past 50 years appear to have been lifestyle factors leading to increased individual exposures. It is not just a matter of time in the sun, but also age at the time of exposure, frequency or intermittency of exposure, and intensity of exposure [79]. The ultraviolet B dose received by the skin depends on the presence of shade and the reflectivity of the surface [106], the clothing worn [107,108], and the use of sunscreens. A major focus of present efforts towards melanoma prevention is a reduction in individual exposure; future trends in melanoma incidence are likely to depend on the success of these efforts.

The long latent period between exposure and clinical diagnosis suggests that any change in ultraviolet exposure will have little effect on incidence or mortality for decades.

4.3. Medical interventions

The impact of a variety of medical interventions must be considered. Most important is the intensity of surveillance for melanoma. As this increases, incidence may increase at least temporarily, as occurred in Scotland, but both case fatality and overall mortality rates may well decline, perhaps substantially. A subgroup of melanomas appear not to be clinically recognizable at an early stage [109], so it may be unrealistic to expect mortality to fall to zero. Nevertheless, the considerable potential of this approach for mortality reduction merits its continued emphasis.

Surveillance for melanoma is not the exclusive domain of medical professionals [61,110–112]. Awareness of melanoma warning signs among the general public may well be the most important component of any campaign for early detection.

Other interventions, such as improved therapy for melanoma or widespread use of effective chemopreventive agents, may well affect future incidence and mortality rates. Improvements in therapy and early detection may decrease mortality appreciably within a few years, and therefore may have a measurable impact long before any effects of change in exposures.

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3. Early detection of melanoma

Lee S. Albert and Arthur J. Sober

1. Introduction

Melanoma is curable when detected early, with 5-year survival ranging from 93% to 100% when Breslow thickness at the time of excision is less than 0.76 mm [1]. Prognosis worsens with increasing thickness, though, and even with advances in melanoma awareness many melanomas are detected too late: 5-year mortality from localized melanoma (American Joint Committee on Cancer Stages I and II) ranges from 15% to 35% in different centers [1].

Suspicious lesions need to be excised at an early and potentially curable stage. In this chapter we will discuss the recognition of melanoma and techniques for screening individuals and populations of individuals for the presence of melanoma.

2. Recognition of melanoma

Melanomas (Figs. 1–3) tend to have four characteristics: asymmetry, border irregularity, color variation, and large diameter. These characteristics can easily be memorized using the mnemonic A-B-C-D and are useful in educating patients and health professionals [2–5]. Any pigmented lesion with these characteristics warrants close evaluation. Asymmetry means that there is no single diagonal that bisects the lesion into two parts that are mirror images of each other. Border irregularity means an indistinct edge or blending of the edge into the surrounding skin. Color variation means that a lesion has several colors in it; classically, black is suggestive of melanoma, but other colors, including white, pink, red, blue, and various shades of brown, may also be present in melanoma. Large diameter means diameter greater than 5 mm, or, alternatively, a lesion exceeding the width of a pencil eraser.

We believe that any pigmented lesion that is growing or changing should be evaluated carefully for the possibility of melanoma. Bleeding, itching, and ulceration also raise suspicion for melanoma.

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Unfortunately, although the A-B-C-D mnemonic is useful and may have good sensitivity and specificity in detecting melanoma [4], neither the A-B-C-D mnemonic nor symptoms such as growing, changing, bleeding, itching, and ulceration yield a definite diagnosis of melanoma in all cases. Many nonmelanoma lesions fit the basic description of melanoma. Melanoma has a very wide differential diagnosis [2,3,5]. A knowledge of this wide differential diagnosis is crucial in detecting melanoma.

Pigmented lesions that must be differentiated from melanoma can be conveniently divided into melanocytic lesions and nonmelanocytic lesions. Melanocytic lesions that must be differentiated include, first and foremost, dysplastic nevi. Dysplastic nevi (Figs. 4–6) are precursors of melanoma and are also markers of increased risk for melanoma in individuals who have them. Like melanoma, dysplastic nevi may have the A-B-C-D characteristics. However, the asymmetry, border irregularity, color variegation, and large diameter are usually present to a lesser degree in dysplastic nevi when compared to invasive melanoma. Often, but not always, an individual with dysplastic nevi has dozens of lesions. If that individual has one lesion in particular that is 'marching out of step' with the others, and showing a greater degree of the A-B-C-D characteristics, then that particular lesion is especially worrisome for melanoma. Other melanocytic lesions in the differential diagnosis of melanoma include the blue nevus (in which brown melanin produced by dermal melanocytes can appear dark or gun metal blue in color), the Spitz nevus (the rapidly growing 'benign juvenile melanoma,' seen more commonly in young people, which has no capacity for metastasis), the congenital nevus (congenital nevi are sometimes quite dark and appear to have an increased risk of eventually undergoing malignant degeneration, especially when giant in size), and the solar lentigo (on sun-exposed skin of older individuals, a solar lentigo can be mistaken for lentigo maligna). Excisional biopsy, when feasible, is the preferred procedure when there is any doubt about the identity of one of these melanocytic lesions [6].

Nonmelanocytic lesions can also be mistaken for melanoma. Seborrheic keratoses (Fig. 7) can be large, irregular, dark, and crusted. Their horn cysts and stuck-on appearance can usually, but not always, differentiate them from melanoma. Flat seborrheic keratoses can be confused with melanoma in situ. Other mimickers of melanoma can include hemangioma, pyogenic granuloma, dermatofibroma, subungual hematoma, tinea nigra, and pigmented basal cell carcinoma. Histologic confirmation is always essential to rule out melanoma if the diagnosis is clinically in doubt.

Amelanotic melanomas lack pigment and therefore can be most difficult to diagnosis. They often are not even suspected at the time of biopsy [7]. The desmoplastic melanoma frequently has an amelanotic clinical presentation. Growth or bleeding of the lesion leads to biopsy, with the amelanotic melanoma eventually being diagnosed by the pathologist. However, because its appearance is clinically nonspecific, it is more difficult for the public and for health professionals to detect early. Maintaining a high index of suspicion

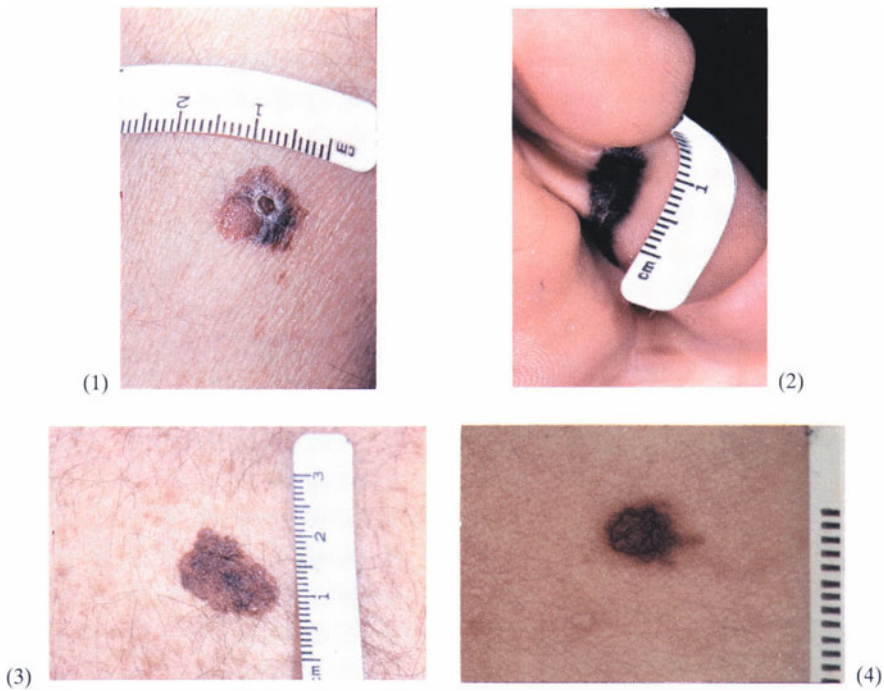

Figure 1. Superficial spreading melanoma, level IV, 1.9 mm thick, on the right arm. Note asymmetry, border irregularity, color variegation, and large diameter (the A-B-C-D characteristics).

Figure 2. Superficial spreading melanoma, level II, 0.4 mm thick, on the left upper back. This thin melanoma still exhibits the A-B-C-D characteristics.

Figure 3. Superficial spreading melanoma, level III, on a toe. This lesion has fuzzy borders, a large diameter, and very dark color, almost black. Even in the absence of color variegation, black color is suggestive of melanoma.

Figure 4. Dysplastic nevi may share the A-B-C-D characteristics of melanoma but usually to a lesser degree. The target or bulls-eye configuration, with a raised darker center and a flat lighter periphery, is frequently noted in dysplastic nevi. Dysplastic nevi may be precursors to melanoma and markers of increased risk. Biopsy may be needed to differentiate dysplastic nevi from melanoma.

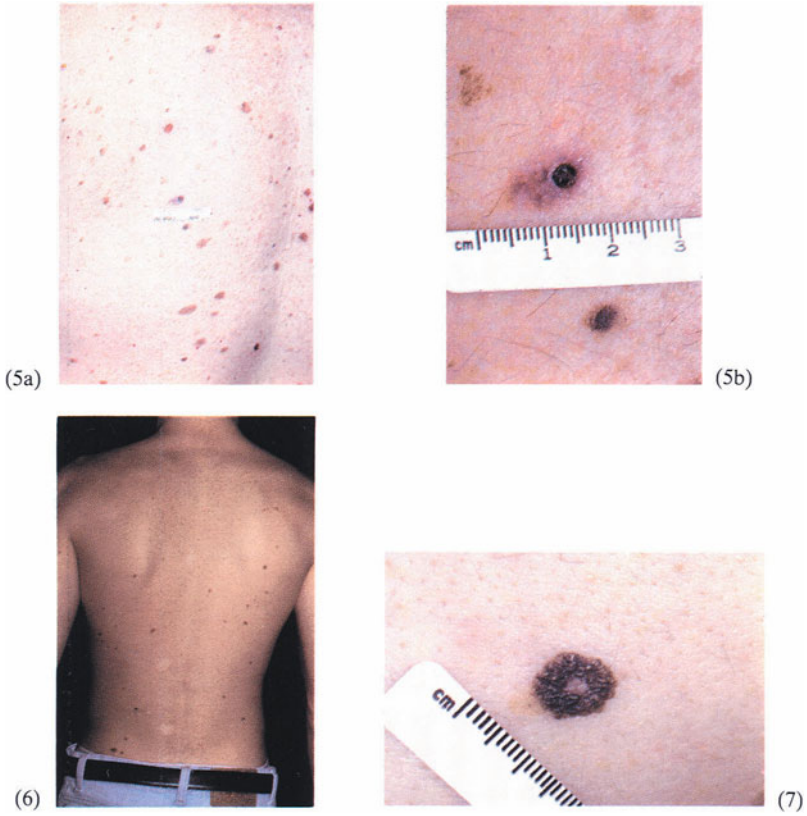

Figure 5. a,b. The man whose back is illustrated in a had multiple dysplastic nevi and seborrheic keratoses. Melanoma level IV (shown in close-up in b) is present in the midst of the dysplastic nevi and seborrheic keratoses. Evaluation of patients with multiple pigmented lesions can be very difficult but is critical in detecting melanoma at an early stage. Note punch biopsy site in 5b.

Figure 6. A man with multiple dysplastic nevi and no history of melanoma. Very prominent mole patterns, with multiple clinically atypical nevi, are not uncommon in individuals who have dysplastic nevi. Halo nevi are also present.

Figure 7. This benign seborrheic keratosis shares the A-B-C-D characteristics of melanoma. The stuck-on appearance and verrucous surface with horn cysts are clues to the correct diagnosis. Biopsy and histologic examination may be necessary in some cases to confirm that a lesion such as this is not melanoma.

and performing biopsies aggressively on rapidly growing lesions can help facilitate earlier detection.

Numerous types of teaching materials are available to educate health professionals and the general public about nevi and melanoma [8]. These illustrative materials can be invaluable aids in learning what 'textbook' melanoma looks like and what signs and symptoms to watch for.

No matter how proficient health care professionals become at detecting melanoma, melanoma will not be detected at an early and potentially curable stage unless patients with signs and symptoms of melanoma come in promptly for examination. Despite extensive publicity campaigns in countries such as the United States and Australia, not all individuals with signs or symptoms of melanoma present promptly for medical evaluation. Hennrikus et al. [9] randomly surveyed individuals in New South Wales, Australia. Of those who had experienced a symptom or sign of melanoma in the previous 5 years, only 57% had consulted a physician; only 32% had consulted a physician promptly (within 5 weeks). Research directed to developing methods of overcoming resistance to seeking medical attention should be encouraged.

3. Screening examination of the general public

One method to help ensure that patients with signs and symptoms of melanoma are seen by a doctor is to initiate melanoma screening examinations. Screening for melanoma can be relatively nonselective, targeting either the general public or patients who present to physicians' offices for any reason. Alternatively, screening for melanoma can target more specifically groups who are felt to be at increased risk for melanoma (such as individuals with congenital moles, individuals with dysplastic nevi, individuals with family members who have melanoma, or older males). Koh et al. have published an excellent review of melanoma and skin cancer screening [10].

The American Academy of Dermatology has sponsored and promoted annual free skin cancer screenings for the public since 1985. Melanomas have been detected during these screenings: During 1986 and 1987 in Massachusetts, 2560 people underwent screening and nine screenees were found to have pathologically confirmed melanoma [11]. Other important skin lesions are also detected during screening. Among the above group of 2560 people, 61 had pathologically confirmed nonmelanoma skin cancer (seven squamous cell and 54 basal cell) and 27 had pathologically confirmed dysplastic nevi [11].

People who are screened are self-selected, raising the concern that the screenees would be people who were concerned about their health but not necessarily those at increased risk for melanoma. Koh et al. [12] surveyed 1116 people who self-referred to the free screenings in Massachusetts in 1987. The screenees were not necessarily persons who were extremely concerned about their health: For instance, the screenees were less likely than

the general population to have had a routine blood pressure check. On the other hand, the screenees did seem to have an abundance of melanoma risk factors. More than 81% had Type I (always burns, never tans) or Type II (burns easily, tans less well than average) skin and 41% had a history of blistering sunburn. Five percent had a family history of melanoma, and 23% had a family history of 'funny moles.' Thirty-six percent of those screened had a changing mole. Therefore, it appeared that an appropriate population was volunteering for screening. Notably, the nine melanomas detected among 2560 screenees in Massachusetts represent an incidence of melanoma seven times that which would be expected by prevalence in a population of this size [11].

Signs and symptoms of melanoma are relatively common and thus lack specificity. Significantly, Hennrikus et al.'s [9] random survey of individuals in New South Wales, Australia revealed that 156 of 1344 respondents (11.9%) had experienced symptoms or signs associated with melanoma within the previous year. These symptoms ranged from itching or tingling nevi (most common) to bleeding or weeping nevi (least common) [9]. With 11.9% of members of the general public having signs or symptoms associated with melanoma in any given year, screening programs would need to examine huge numbers of people in order to check all those who experience symptoms. This raises the issue of the limitations of free screening: Koh et al. [5] reported that in the first few years after screening was begun in 1985, 358,000 individuals participated in skin checks. This compares with a population of over 250 million in the United States: The vast majority of people, including many with melanoma risk factors, are not going to be reached by free annual screenings. This limitation applies to other countries as well. Of course, the publicity generated by screening efforts could certainly increase the public's awareness of nevi and motivate people with signs or symptoms of melanoma to see their providers of health care at other times.

4. Screening office patients

Another way to detect melanoma earlier would be in the office of the primary health care professional or the dermatologist. Since the majority of Americans have regular medical examinations, and since a substantial minority have a regular dermatologist [12], there is great potential for early melanoma detection through periodic skin checks by these care providers. If full skin examinations were done regardless of the patient's presenting complaint, melanoma or other important skin lesions potentially could be detected as an incidental finding. Lee et al. offered full skin checks to 874 new dermatology patients; 81% agreed to the examination [13]. Histologically confirmed melanoma was found in one, lentigo maligna in three, and histologically confirmed dysplastic nevi were found in 17. Other important findings included nonmelanoma skin cancers in 20 patients and actinic

keratoses in 64. Lookinbill similarly offered complete skin examination on 1157 new dermatology patients; 96% agreed to the examination [14]. He detected and confirmed histologically melanoma in one patient. Kaposi's sarcoma was detected in one patient and basal cell carcinomas in 20. Twenty-five patients were clinically suspected to have dysplastic nevi. Both Lee et al. [13] and Lookinbill [14] found a higher incidence of significant incidental findings in older compared to younger patients (although more young adults than older adults in Lookinbill's group had dysplastic nevi). The findings of Lee et al., of Lookinbill, and of other similar studies indicate that total body skin examination in all patients who present to a dermatologist, regardless of the presenting complaint, can lead to incidental detection of important skin lesions, including melanoma. Of course, full skin examinations do have a cost in terms of both the patient's and physician's time, and the potential for psychic discomfort in modest patients. Controversy persists and total body skin examinations have not become a standard of care.

5. Screening target groups: Dysplastic Nevi

Another approach to detecting melanoma at an early stage utilizes screening exams and periodic follow-up exams for selected individuals who are felt to be at high risk for melanoma. Rhodes et al. [15] have reviewed the groups at high risk for melanoma who might be targeted for screening: Risk factors include a persistently changed or changing mole; adulthood; one or more large or irregular pigmented lesions (including dysplastic moles, which are both melanoma precursors and also markers of increased melanoma risk, and lentigo maligna); congenital moles; Caucasian race; previous cutaneous melanoma; cutaneous melanoma in parents, children, or siblings; immunosuppression; sun sensitivity; and excessive sun exposure. MacKie et al. [16] created a risk factor flow chart based on the presence or absence of four melanoma risk factors (greater than 20 nevi, presence of freckling, presence of atypical nevi, and history of sunburns). They suggested that the risk-factor flow chart might prove useful for choosing candidates for screening.

The targeted screening approach has been used most frequently and with greatest success in the management of individuals who have a personal or family history of dysplastic nevi or melanoma. The Pigmented Lesion Clinic at Massachusetts General Hospital is a model for the screening and management of these individuals. When an individual comes in for evaluation, he or she is examined from head to toe, with close attention not only to sun-exposed but also to sun-protected areas. Clinical features of nevi and melanoma are reviewed with each patient, with illustrative examples. The use of sun protection measures (sunblock or protective clothing) and the need to avoid excess sun are discussed; special emphasis is placed on avoiding sunburn. If no remarkable pigmented lesions are found, the patient is so informed and told to return if any lesion changes or if new lesions develop.

Any patient with a lesion suspicious for melanoma is referred for immediate excision, the same day if possible. Patients who are found to have at least one clinically atypical nevus are educated in regard to dysplastic nevi as well as melanoma. The need for close follow-up to facilitate early detection is discussed. Patients are instructed to perform bimonthly home examinations of their entire cutaneous surface, usually with the help of a mirror and/or a family member. Routine examination by the Pigmented Lesion Clinic or by the patient's dermatologist on an annual basis (or even more frequently if patients have a strong personal or family history of melanoma) are suggested.

Some authors, such as Cohen et al. [17], advocate a very aggressive surgical approach to the management of patients with dysplastic nevi. They recommend a very low threshold for prophylactic removal of clinically atypical nevi, reasoning that simple observation might permit the progression of a dysplastic nevus to an incurable melanoma. They note, too, that patients are all too easily lost to follow-up. Cohen et al. prophylactically removed an average of 17.7 pigmented lesions from a selected group of 190 melanoma and nonmelanoma patients during a 4-year period: among the lesions removed were 12 clinically unsuspected in situ melanomas and three clinically unsuspected invasive melanomas.

This aggressive approach to dysplastic nevi has its drawbacks. In patients with dysplastic nevi, who frequently have multiple lesions, it is estimated that up to several dozen nevi would have to be removed to prevent one melanoma [18]. Also, patients with dysplastic nevi continue to develop new nevi throughout life, and they can develop melanoma on previously normal skin, not just in dysplastic nevi. Barnes and Nordlund [19] reported the case of a man who had over 150 nevi removed prophylactically; over the next 7 years, he continued to develop new dysplastic nevi. Therefore, even if all their moles were taken out at one point in time, patients could still develop dysplastic nevi and melanoma, and would still need close follow-up.

There is no question that a strong family history of melanoma increases the risk of melanoma developing in individuals with dysplastic nevi. In the setting of familial melanoma, melanoma and dysplastic nevi appear to be inherited on an autosomal dominant basis [20,21]. Individuals who have dysplastic nevi and come from melanoma-prone families (with at least two other family members having melanoma) have a 3% annual incidence of melanoma and a lifetime incidence of melanoma that approaches 100% [22].

What is the risk of melanoma developing in patients with dysplastic nevi (DN) and a negative family history of melanoma? Halpern et al. [23] examined 105 patients with nonfamilial melanoma and 181 controls. They detected clinically dysplastic nevi in 39% of the melanoma patients and 7% in the control patients. The odds ratio for dysplastic nevi in melanoma patients vs. controls was 8.8, adjusted to 6.8 after correcting for sex, age, hair and eye color, freckling, and benign nevus counts. This increased risk of melanoma associated with DN outside the familial melanoma setting confirms the importance of closely following DN patients regardless of their family history.

The recommendation of the Pigmented Lesion Clinic is that all first degree relatives of individuals with dysplastic nevi or melanoma receive a screening evaluation. Unfortunately, although most dermatologists suggest screening of family members of melanoma patients, relatives often do not come in for examination [24].

Dysplastic nevi that seem to be occurring on a sporadic basis may well turn out to be familial when blood relatives are examined. Crijns et al. [25] examined 125 blood relatives of 31 individuals with dysplastic nevi who did not report a family history of dysplastic nevi. When examined, 13% of the parents and 36% of the siblings had dysplastic nevi (in comparison, only 6.5% of a control group of new dermatology patients and nursing home residents had dysplastic nevi). The blood relatives had a fourfold increased likelihood of having dysplastic nevi compared to controls. Sixty percent of the patients with supposedly sporadic dysplastic nevi turned out to have relatives with dysplastic nevi. The bottom line is without direct examination, it may be misleading to accept a patient's statement that no family members have dysplastic nevi.

The practice of bringing in family members for examination has proved fruitful at the Pigmented Lesion Clinic at Massachusetts General Hospital. The experience in this area has recently been published, demonstrating that screening and close follow-up of patients with dysplastic nevi and of their blood relatives led to detection of melanoma at an early and potentially curable stage [26].

The effectiveness of surveillance programs like the Pigmented Lesion Clinic in detecting melanoma is becoming apparent. Tiersten et al. [27] followed 357 patients with dysplastic nevi for an average of 49 months, and detected 10 invasive melanomas and eight in situ melanomas in 17 patients.

Surveillance programs appear to be effective not only in detecting melanoma, but also in detecting it at an early and potentially curable stage. All of the melanomas detected by Tiersten et al. [27] were less than 0.86 mm thick. Melanomas detected by close surveillance of individuals with dysplastic nevi or melanoma, and of their families, are likely to be excised with a lower thickness and a better prognosis. Masri et al. [28] compared the first melanoma detected in a family with melanomas subsequently detected by screening and surveillance of blood relatives. The first melanoma detected in a family had an average thickness of 1.48 mm, compared with an average thickness of just 0.52 mm for melanomas subsequently detected by family screening and surveillance. Similarly, Vasen et al. [29] looked at melanoma-prone families and compared melanomas detected before screening was initiated with melanomas subsequently detected by screening and surveillance of family member Melanomas detected before screening was begun had an average thickness of 1.75 mm. In comparison, the average thickness of melanomas detected by initial screening of family members was 0.80 mm, and the average thickness of melanomas detected by follow-up surveillance of family members was 0.54 mm. Screening programs thus helped to reduce the thickness of melanoma and to increase the probability of cure.

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4. The role of microscopic evaluation in the management of cutaneous melanoma

Alistair J. Cochran, Scott Binder, and Fabrizio Remotti

1. Introduction

Microscopic evaluation of excised tumor tissues is essential in the management of patients with cutaneous malignant melanoma. Assessment of a putative primary tumor allows confirmation that the tumor is melanocyte derived, that it is malignant and primary in the site, provides indications of the likelihood that it will metastasize, and confirms the completeness of excision. Evaluation of tissues from sites that, on clinical grounds, are regarded as harboring metastatic tumor allows confirmation that tumor is present and that the tumor is metastatic melanoma rather than a metastasis from a second, unsuspected, nonmelanocytic primary tumor. In the case of melanoma spread to the regional lymph nodes, the extent of nodal involvement by tumor provides information on the likelihood that further metastases will occur after lymphadenectomy.

The need for histologic evaluation of all pigmented tumors to establish an accurate diagnosis would seem self-evident. Even very experienced clinical observers encounter difficulty in diagnosing pigmented lesions by clinical evaluation alone. Pigmented lesions of the skin are best evaluated by considering carefully their appearances on inspection (preferably using low to medium magnification, provided by a hand lens or dissecting microscope), by considering the patient's age and sex, and the site of the lesion, and obtaining a careful history of the nature, extent, and timing of any alterations in shape, size, or color. The microscopic appearances must be considered in the light of information on the gross appearances and history of the lesion. It is therefore essential that clinical data be provided to the reporting pathologist.

The nature of the biopsy and the manner in which it is obtained are also critical. Material for diagnosis should be obtained by excision biopsy, wherever possible, and great care should be taken not to compress the tissues with forceps or other crushing instruments. Cautery and chemical sclerosants are to be avoided, since they distort residual tumor, making evaluation of subsequently reexcised tissue difficult. Optimal fixation requires that the tissue be placed in an adequate volume ($\times 10$–20) of fixative

L. Nathanson (ed.), Current Research and Clinical Management of Melanoma.

immediately after its removal and be allowed to remain in fixative long enough to allow complete penetration of the tissues. Penetration time obviously relates to biopsy size, and this should be considered in balancing the need for a rapid diagnosis against the need for complete tissue evaluation.

These are among the most important comments in this chapter. It will be easy to dismiss them as the unreasonable obsessions of pathologists who do not understand the pressures of a busy clinical practice. Perhaps, but problems of underdiagnosis and undertreatment, or of overdiagnosis and overtreatment, are often the result of insufficient history or errors in the interpretation of tissues that are technically suboptimal.

The great majority of melanocytic lesions can be identified on the basis of a hematoxylin and eosin stained slide. The differential diagnosis of melanoma, especially of amelanotic melanoma, is, however, substantial, and where doubt exists immunohistochemistry and electron microscopy can often provide assistance.

2. Microscopic diagnosis of primary melanoma

2.1. Light microscopy of conventionally stained sections

The pathologist evaluating a pigmented lesion attempts to answer a series of questions concerning whether or not the lesion is of melanocytic origin and whether it is malignant or benign. If it is a malignant melanoma, is it primary or metastatic, and to what subclassification does it belong. What is the likely clinical outcome and has the tumor been excised? It is of some assistance, in attempting to answer these critical questions, to consider the nature and origin of cutaneous malignant melanoma, a malignant tumor that with few exceptions (see section on melanoma and nevi below), arises from the melanocytes of the basal layer of the epidermis and less often from those of associated skin appendages. Primary melanomas are generally greater than 6 mm in diameter, while most acquired nevi are smaller. Overlap is inevitable. Dysplastic nevi and some congenital nevi can be as large as a typical primary melanoma, and some can be much larger. Nonetheless, large pigmented lesions require careful attention, especially if there is a history of alteration in size, profile, or pigmentation.

2.1.1. Is the lesion melanocyte derived? Characteristic of the melanocyte is the ability to synthesize melanins from tyrosine using enzymes, including DOPA-oxidase. This synthesis takes place on characteristic organelles, the melanosomes. A fully melanized melanosome is just visible on light microscopy as a distinct and separate structure, and the presence of intracytoplasmic melanin in this pattern is proof positive that a cell is melanogenic. After full melanization melanosomes are shed by the cells that generate them and are taken up by adjacent cells, including keratinocytes and

macrophages, in which they often become aggregated, forming readily visible masses that may be 2–3 μm in diameter. Such aggregated melanosomes do not, by themselves, indicate that a cell is generating melanins, as such structures may be acquired by endocytosis. The presence of melanophages (melanin-containing macrophages) indicates that there are or have been melanogenic cells in the vicinity or in tissue connected to the site by lymphatic drainage channels.

Pigments other than melanin are brown and may be present in the cell cytoplasm in a microparticulate form. That the pigment is melanin is determined by its failure to react with special stains for iron and its removal from the section by bleaching agents such as potassium permanganate. In the absence of melanin synthesis (more common in metastases than in primaries), immunohistochemistry and electron microscopy are of assistance. These are discussed in detail below. An alternative is to demonstrate the presence of the enzyme DOPA-oxidase by incubating tissue slices with colorless tyrosine and in the presence of DOPA-oxidase, observing the generation of brown-pigmented melanins (DOPA reaction) [1]. Because of the availability of immunohistochemistry, this approach is now less often employed.

The presence of eosinophilic intranuclear inclusions (actually cytoplasmic invaginations) should raise the possibility that a lesion is melanocytic, but is not diagnostic, as other classes of cells may show a similar appearance [2].

2.1.2. Is the lesion benign or malignant? At its most basic this consists of determining whether or not the histologic pattern of the lesion and the cytology of the cells that comprise it differ significantly from those of benign nevi. The majority of benign nevi, especially those of acquired type (absent at birth, developing at and beyond puberty), are small lesions that are uniformly pigmented, hair-bearing, well circumscribed, and when fully developed are symmetrical on vertical section through the center point. These characteristics do *not* describe dysplastic nevi, and these important lesions are discussed in the section devoted to precursor lesions. Melanomas are generally larger, and because most have abnormal melanocytes (increased in frequency, pleomorphic, and possibly suprabasal) in the epidermis adjacent to the invasive component (radial growth phase or dysplastic nevus) and show variable pigmentation from area to area within the tumor, they are often irregular in outline, poorly circumscribed, and strikingly asymmetrical.

Epidermal alterations are common in the skin adjacent to primary melanoma. In the absence of trauma benign nevi are scarcely ever ulcerated, while ulceration is a common accompaniment of primary melanoma. Actively growing nevi may be associated with mild to moderate epidermal hyperplasia, an pseudoepitheliomatous hyperplasia is characteristic of actively growing Spitz nevus. The degree of hyperplasia is (with the exception of the Spitz nevus) seldom as marked as the acanthosis that is usual in the epidermis of

superficial spreading melanoma. By contrast the epidermis over lentigo maligna and lentigo maligna melanoma is usually thin.

In both actively growing nevi and primary melanoma, there is an increased frequency of melanocytes associated with the basal layer of the epidermis. This increase takes two forms: an increase in single melanocytes and the formation of colonies (nests) of cells that appear suspended from the underside of the epidermis. The difference is that in nondysplastic benign nevi the melanocytes, while possibly enlarged, show neither pleomorphism nor nuclear atypia, characteristics that are commonly seen in malignant melanoma (and dysplastic nevi). In benign nevi the nests tend to be evenly distributed, are of regular size, and frequently arise on the tips and shoulders of the rete ridges (Fig. 1). By contrast, in melanoma the nests are irregular in their distribution, size, and shape; may fuse one to the other; and show no special association with the rete ridges. A further feature of note is that in fully evolved benign nevi the epidermal and subjacent dermal components are usually coterminous, while this is not so in the majority of melanomas, the radial growth phase being, by definition, not underlain by a dermal component. The presence of melanocytes singly and in small groups in the suprabasal epidermis (pagetoid appearance) is characteristic of superficial spreading melanoma (Fig. 2). Occasional single suprabasal melanocytes may be seen in the epidermis overlying actively growing nevi, genital nevi of young women, nevi of palms and soles, some congenital nevi, especially those of infants, and Spitz nevi during active junctional growth. We have never seen a fully developed pagetoid appearance in such lesions. Care must be taken not to misidentify as melanocytic keratinocytes in which the cytoplasm has shrunk away from the nucleus. The separation can usually be made by identifying intracellular bridges at the border of the keratinocytes.

A consideration of tumor cell cytology is critical in separating nevi from melanomas. In most nevi the cells that are shed into the dermis from the junctional nests are moderate sized, round to epithelioid with eosinophilic cytoplasm, small centrally located, round to oval nuclei that are lightly to moderately basophilic and that have one or more small basophilic nucleoli. As these cells pass deeper into the dermis, they lose the capacity to synthesize melanin, become smaller, contain reduced amounts of cytoplasm, and develop smaller featureless highly basophilic nuclei (nevocytic differentiation) [3]. The cells of melanoma may resemble those of nevi (nevocytoid melanoma) [4], but in most instances are moderate to large in size; epithelioid more often than spindle-shaped; have abundant cytoplasm that is often amphophilic (due to increased amounts of RNA); have nuclei that vary in size (leading to a high nucleo-cytoplasmic ratio), contour, intracellular position, and basophilia; and show nucleoli that are usually large and often eosinophilic. In contrast to the cellular maturation characteristic of nevi, the cells in the deeper portions of most melanomas differ little from those in the junctional and immediately subepidermal positions.

With the exception of the alterations associated with maturation as

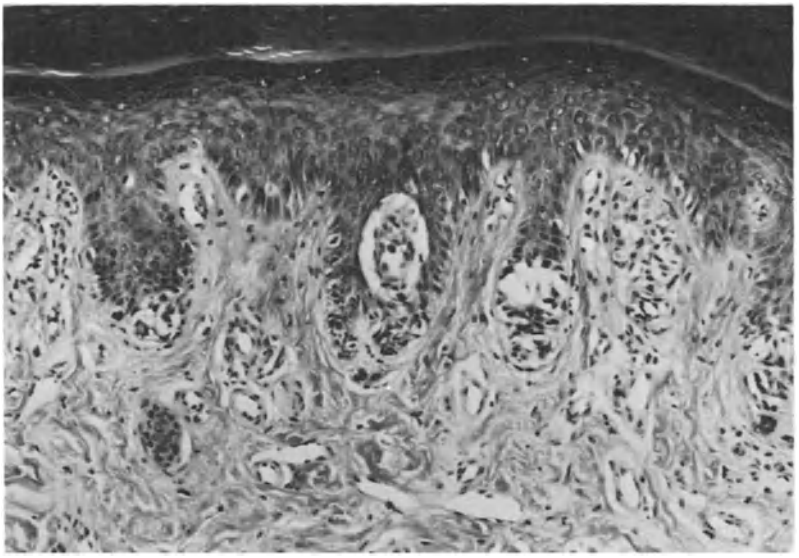

Figure 1. Superficial compound nevus of sole of foot, regularly shaped showing melanocyte colonies (nests) located at and close to the tips of rete ridges. Hematoxylin and eosin (×240).

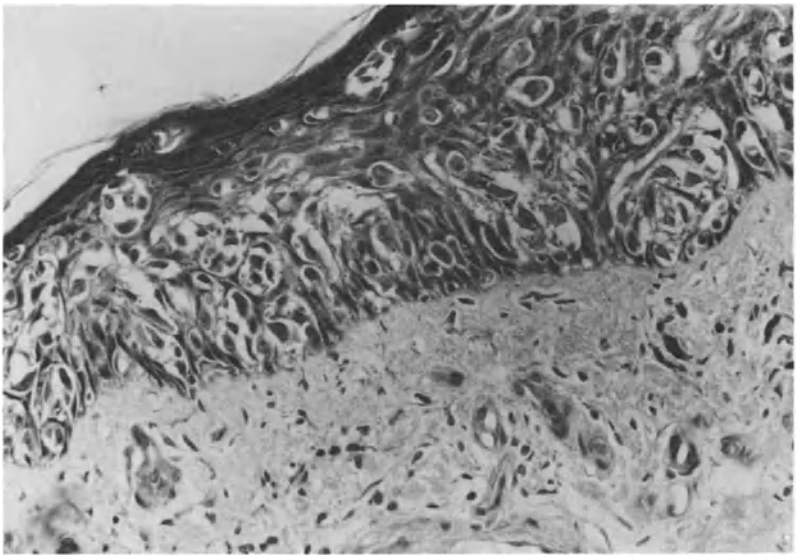

Figure 2. Malignant melanocytes present singly and in small groups in the suprabasal epidermis (pagetoid appearance) of the radial growth phase of a malignant melanoma of superficial spreading type. Hematoxylin and eosin (×420).

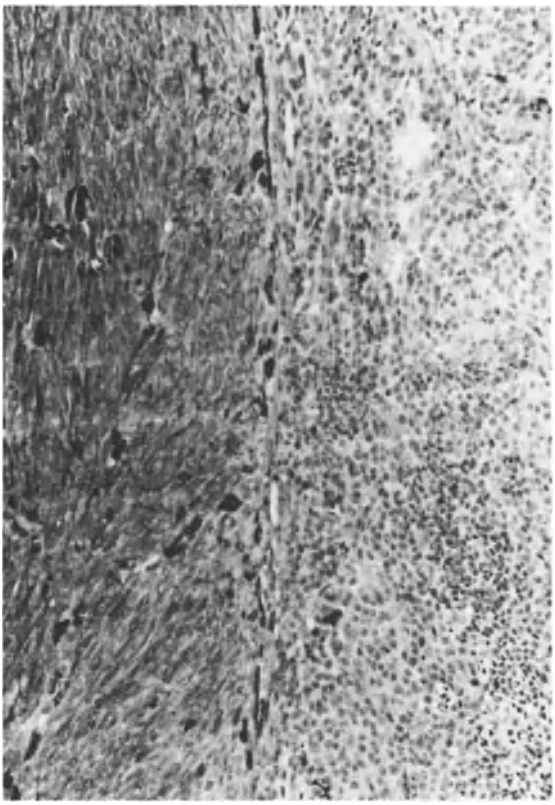

Figure 3. Intralesional transformation. This is an ocular melanoma in which there were two readily separable populations of tumor cells. That on the left was large spindle celled and heavily melanized, in contrast to that on the right, which was hypomelanotic and comprised of small round to oval cells. Hematoxylin and eosin (×280).

nevocytes migrate into the dermis and the presence of occasional giant cells, the cells of benign nevi are remarkably homogeneous. By contrast, the cells of many melanomas are quite heterogeneous. This is strikingly seen when an evolving melanoma abuts or infiltrates an antecedent nevus. The process, known as intralesional transformation, represents a striking form of cellular heterogeneity. In this one or more subpopulations of cells (that may be truly clonal) that are recognizably different from the remainder of the tumor expand as separate nodules within the main tumor mass (Fig. 3). Recognizable differences include cell size variation, variations in the capacity to generate melanins, mitotic rate, and capacity to induce a lymphohistiocytic response. In a study of ocular melanoma, we have recently shown that these striking alterations in morphology are accompanied by fundamental differences in tumor cell biochemistry, manifested in our study as alterations

in the expression of gangliosides from a profile characteristic of normal melanocytes to that of metastatic melanoma cells [5]. These alterations were paralleled by increased metastatic propensity.

In nevi the cells at the epidermo-dermal interface and those in the upper dermis often synthesize melanin and may show mitotic activity, while those located more deeply are generally devoid of melanin and rarely show mitotic activity. There is evidence that to generate melanins the cells of benign melanocytic nevi require the costimulus of ultraviolet light, while the relatively autonomous melanoma cells can generate melanins in the absence of extraneous stimuli. The presence of mitotic activity in the deeper area of a nevus-like lesion calls for a stringent review to exclude that it is a minimal deviation melanoma [4,6]. In melanomas pigmentation is common in tumor cells at all levels of the dermis and mitoses are seen in tumor cells in the superficial and deep dermis. Atypical mitoses are not uncommon in melanomas but are exceedingly rare in nevi. Single cell necrosis (apoptosis) is not common in melanocytic lesions, but if present usually indicates a melanoma. In contrast to metastatic melanoma, large areas of necrosis are seldom seen in primary melanomas.

The dermal cells of nevi in the earlier stages of their evolution may be present as discrete groups (packets) or may be singly dispersed. Most melanomas invade the dermis as sheets of cells or in packets of cells that abut each other, creating an appearance that simulates a sheet of cells. A feature of the highest importance is that once a primary melanoma begins to develop the junctional component persists, and there is rarely if ever a 'grenz' zone, a band of stroma between the epidermal and dermal components. The only exception to this assertion is an occasional grenz-like zone of fibrosis where the top of a melanoma has been removed by ulceration or a superficial shave and the lesion has reepithelialized. By contrast in nevi, the growth period of which is usually self-limiting, grenz zones are common as the nevus evolves from a compound to a purely intradermal phase. At the deep limit of their extension, both nevi and melanomas may show a pushing edge or may raggedly infiltrate the dermal stroma. In nevi the tumor cells tend to lie between the dermal collagen fibers, while in melanomas infiltrating tumor cells often destroy such fibers.

As the nevus ages individual cells and small groups of cells become invested by fine fibrous tissue, a process that eventually leads to total involution of the nevus. Fibrosis may also occur in melanomas, most commonly in areas of regression. Melanoma-associated antigens are recognized as foreign by the immune system, and an immune reaction is mounted against the tumor cells that express them. This destroys some or occasionally all the tumor cells. Activated lymphocytes and dying tumor cells release cytokines, some of which crossreact with adjacent fibroblasts, leading to the local formation of new fibrous tissue that, in its early stages, may be strikingly vascular. Where the destroyed tumor cells contained melanin, this pigment is released and is present free in the scar tissue or in melanophages. Another form of

fibrosis is seen in the melanoma variant known as desmoplastic melanoma [7], in which the tumor cells generate cytokines that directly stimulate adjacent fibroblasts. In desmoplastic melanoma the fibrosis is pericellular, but as the ratio of fibrosis to tumor cells may be high, it can be extremely difficult to identify tumor cells in a mass of exuberant fibrosis.

Nevi (other than dysplastic nevi) seldom evoke the kind of lymphohistiocytic infiltrate that is characteristic of melanoma, especially in the early stages of its evolution. This infiltrate, which typically is comprised of T lymphocytes showing varying degrees of activation, dermal dendritic cells, occasional macrophages, and B lymphocytes [8], may vary in density from light to heavy and is most prominent in response to melanoma in situ and superficially invasive melanoma. In the great majority of tumors the infiltrate forms an investing sheath that sits immediately deep and peripheral to the outermost tumor cells, but in a minority of tumors the infiltrate penetrates the substance of the tumor, coming to lie between the individual tumor cells. The invasion of the tumor by lymphocytes appears to indicate a separate biological situation and may favorably influence the clinical outcome [9,10].

2.1.3. Is the melanoma in situ or invasive? This is a determination of the highest importance as true melanoma in situ does not metastasize. It is sometimes difficult to be sure whether or not microinvasion has occurred, especially where there is a well-developed 'lichenoid' lymphoid infiltrate, where the epidermis shows complex hyperplasia (making it difficult to be sure that the section is cut vertically), and where the specimen has been crushed or improperly fixed. It is useful to examine multiple levels, and very occasionally it may be appropriate to resort to immunohistochemistry to determine the position of tumor cells relative to the basement membrane. Not all melanomas in situ show pagetoid penetration of the overlying epidermis, but the presence of pagetoid change strongly favors a diagnosis of melanoma. The implications of the separation of melanoma in situ from microinvasive melanoma have been lessened by the observation by Clark et al. [11] that melanomas do not acquire the capacity to metastasize until they have formed at least one expansile colony in the dermis. Thus the presence of single melanoma cells free in the upper papillary dermis may be regarded as a later stage of the radial growth phase (Fig. 4). While it remains desirable to separate these two 'entities,' they are probably best regarded as different stages of the evolutionary process by which transformed melanocytes progress to the stage of significant local invasiveness and the capacity to metastasize. They are both cured by local excision.

Melanomas invade by direct extension, first into the papillary dermis and then into the reticular dermis and subcutis. During this invasion they infiltrate and may destroy skin appendages, antecedent nevi, and cutaneous nerves (neurotropism, characteristic of desmoplastic melanoma; Fig. 5). Involvement of cutaneous lymphatics and blood vessels are important observations

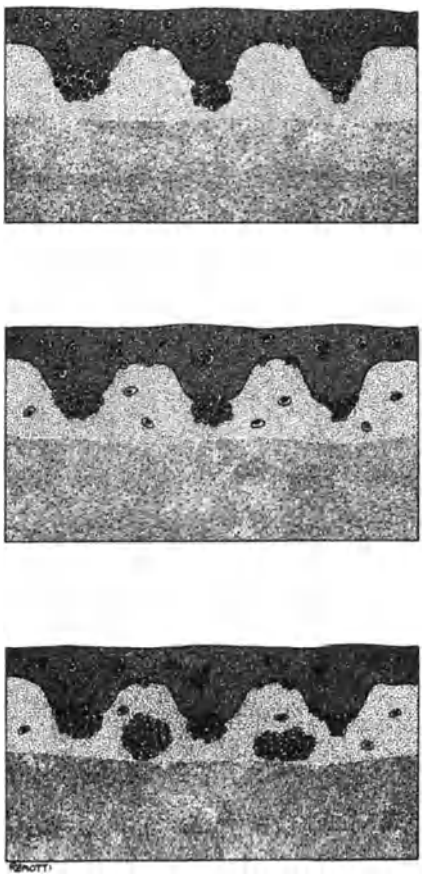

Figure 4. Melanoma-in-situ vs. early invasion. The top panel shows melanoma in situ with cytologically abnormal melanocytes in the suprabasal epidermis and at the epidermo-dermal junction. This lesion has no potential to metastasize. The middle panel shows, in addition to the above, single melanoma cells set in a fibrotically expanded papillary dermis. In the absence of expansile colonies in the dermis, this is regarded as the late stage of the radial growth phase and has no potential for metastatic spread. The lower panel shows microinvasion with single cells and two expansile colonies of melanoma cells in the papillary dermis. This stage of melanoma evolution carries a real, but low, risk of metastasis.

because of their relationship to the generation of metastases. Microsatellites are colonies of tumor cells deep in the dermis and apparently detached from the main tumor mass, that have been associated by some authors with an unfavorable prognosis [12]. Some microsatellites are likely to be endolymphatic or endovascular tumor deposits, while others may represent advancing columns of tumor cells tenuously attached to the main tumor mass.

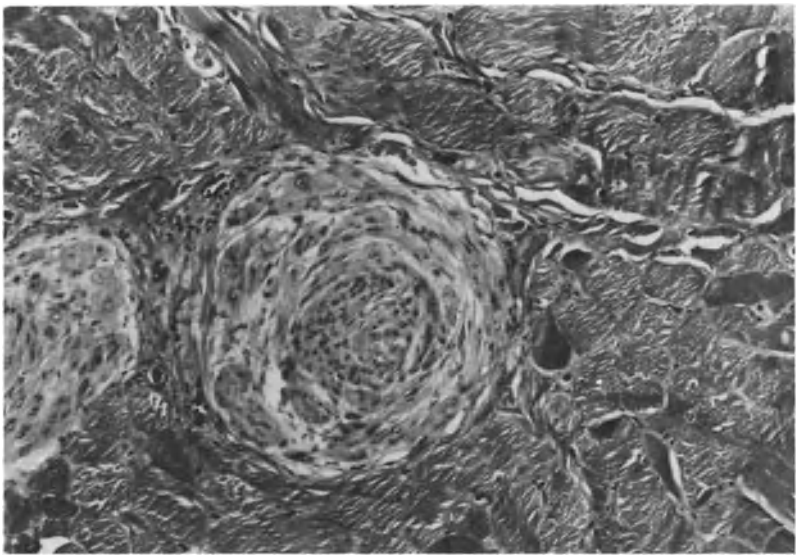

Figure 5. Neurotropism. A cutaneous nerve is ensheathed by spindle-celled melanoma. This is most commonly seen in desmoplastic melanoma. Hematoxylin and eosin (×220).

2.2. *Immunohistochemistry*

The development of the techniques of immunohistochemistry and of antibodies against epitopes that are at least relatively specific for cells of an individual lineage (*differentiation antigens*) has greatly facilitated the identification of the histogenesis of poorly differentiated tumors. This approach has been widely applied to the identification of tumors of melanocytic origin, particularly those that synthesize minimal melanin or are truly amelanotic.

The initial differentiation antigen that was evaluated was S-100 protein [13–15], a dimeric protein extracted from bovine brain and initially evaluated for its expression in the neural tissues of many different species (reviewed Cochran and Wen [16]). S-100 protein was identified in cells of melanocytic lineage by our own group [13], and in succeeding years we and others developed it for use as a practical diagnostic agent [14,15]. S-100 protein is a highly sensitive marker for cells of melanocytic lineage, being present in greater than 99% of such tumors. It is, however, relatively nonspecific, being present in a wide range of cells, including Schwann cells, lymphoid dendritic cells, chondrocytes, myoepithelial cells, etc. Despite this, S-100 protein remains a mainstay of the immunodiagnosis of melanocytic tumors, especially when used as part of a 'package' of different reagents (see below). The two monomers of S-100 protein are present to varying degrees in

Table 1. Panel of antibodies used in the identification of melanocytic tumors

Reagent	Source
S-100 protein	DAKO, Carpinteria, CA
HMB-45	ENZO, New York, NY
* Polyvalent anti-cytokeratin	DAKO, Carpinteria, CA
** Leukocyte common antigen	DAKO, Carpinteria, CA

* To detect epithelial tumors.
** To detect lymphomas.

different tissues, and the availability of antibodies to S-100α and S-100β should increase the specificity of this approach.

More recently monoclonal antibodies to other differentiation antigens have become available, and we have found two, in particular, of considerable practical value. These are the antibodies NKI/C3 [17] and HMB45 [18]. Both reagents are more specific than S-100 protein, although by no means absolutely specific. On the other hand, neither is as sensitive as S-100 protein. All three reagents can be used against both fixed, paraffin-embedded materials and frozen sections, though both S-100 protein and HMB45 are best used with fixed material [19].

In practice we use a panel of antibodies (Table 1) to attempt positive identification of cells of melanocytic origin (antibodies 1–3) and to exclude that they are derived from hemopoietic (antibody 4) or epithelial (antibody 3) sources.

Interpretation of immunohistochemical results must be keyed to all available clinical and pathological information. A diagnosis should not be made on immunohistochemistry alone, and immunohistochemical findings should generally not overturn a diagnosis made on a clinicopathologic basis. Numerous pitfalls are known that render immunodiagnosis difficult, and no doubt others remain to be identified. The techniques are not, in themselves, difficult to perform but require practice and facility to achieve reliable and consistent results. Reagents have a definite shelf life and need to be stored under optimal conditions to maintain peak performance. A problem is that there is a temptation to continue using expensive reagents that are time expired. There is therefore an argument for having this kind of work undertaken in laboratories that deal with large volumes of tests.

In addition to problems of a technical nature, the reagents available, being less than totally specific, yield curious 'cross-reactions,' and individuals undertaking such evaluation must be aware of these. For example S-100 protein is found in certain minority subclasses of lymphocytes (the cells that are the basis of hairy cell leukemia) [20], and while melanoma cells do not contain the commonly detected classes of cytokeratins, they may express small amounts of low molecular weight keratins. Many new reagents are

under development, and it is to be hoped that these will combine the desirable characteristics of high sensitivity and high specificity.

In the evaluation of melanocytic tumors, immunohistochemistry has mainly been used to separate these tumors from nonmelanocytic tumors. An equally desirable goal is to separate benign from malignant tumors. Indications that this may be possible come from studies of a different class of antigen, known as *progression associated antigens*. These are antigens that are selectively expressed at different stages of the evolution of cells from normal melanocyte through nevus cells of various types to the cells of primary and metastatic melanoma. Epitopes considered to fall in this group include growth factor receptors (e.g., nerve growth factor receptor), growth factors, adhesion molecules (e.g., VLA-2, ICAM-1), HLA-DR, DQ, DP, and the cell cycle-associated molecule Ki67. All of these have some value in separating melanoma and nevi, but with the exception of the HLA-molecules, most cannot be used on fixed material, a major impediment to their routine use [21].

In addition to its well-established use in determining that a tumor is of melanocytic lineage and an evolving employment in the separation of benign and malignant melanocytic tumors, immunohistochemistry can also be employed very effectively to detect occult tumor cells, tumor cells that are not visible on conventional assessment, in, for example, the regional lymph nodes or bone marrow [22,23]. Other applications are to assist in the microstaging of primary melanoma and to evaluate the histogenesis of single cells in effusions.

2.3. Electron microscopy

Although the development of sensitive and relatively specific immunohistochemical markers for the diagnosis of malignant melanoma has diminished the importance of electron microscopy, ultrastructural examination still has a definite role in the characterization of melanocytic tumors [24,25]. Malignant melanoma must be considered in the differential diagnosis of any anaplastic or undifferentiated tumor, and there are times when even a panel of antibodies will fail to distinguish the origin of a tumor. In these instances, electron microscopy remains the only hope for a clear-cut diagnosis. We encounter several such problem cases each year. The differential in these instances is usually melanoma vs. malignant peripheral nerve sheath tumor or melanoma vs. poorly differentiated carcinoma (including neuroendocrine carcinoma) or sarcoma.

The diagnosis of malignant melanoma is usually established by electron microscopy by demonstrating organelles of a characteristic morphology, called melanosomes, in the tumor cells [25]. These organelles have a characteristic internal structure (Fig. 6), but occasionally it may be difficult to distinguish the melanosome from other organelles that may resemble it (e.g., lysosomes, Weibel-Palade bodies, etc.). Furthermore, cells other than

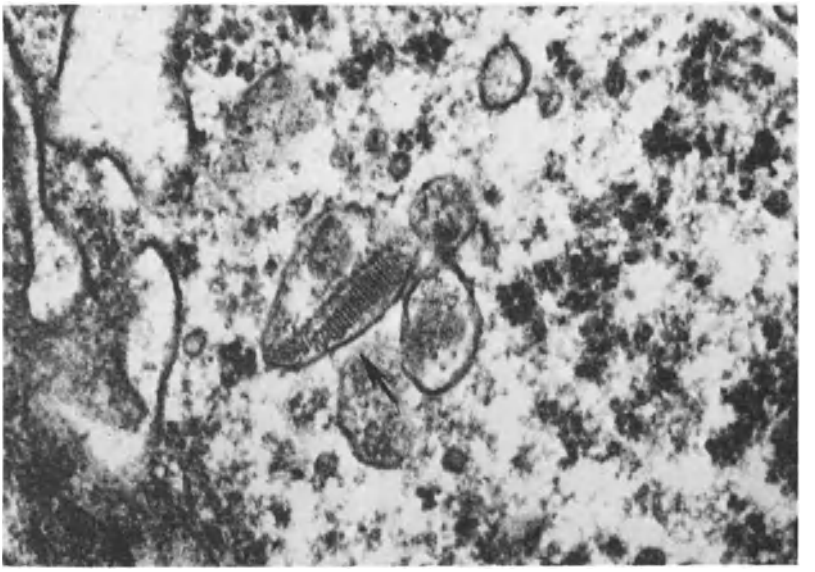

Figure 6. High magnification of a premelanosome (Stage II; arrow). Note the characteristic transversely striated internal substructure (×120,000). Courtesy of Dr. S. Bhuta, UCLA.

melanocytes may contain melanosomes. Thus, sampling effects and technical considerations may interfere with the diagnosis of a particular case. Although the substructure of melanosomes is relatively stable and paraffin blocks are often adequate for a diagnostic assessment, suboptimal preparations make the identification of melanosomes impossible. It is certainly best if fresh tissue can be subjected to the special fixation necessary for the effective preservations of ultrastructural features, and clinicians can ensure that material is prepared in this way by prearranging the reception of specimens for special handling and providing an adequate history to their pathologist colleagues. Examination of many sections may be necessary in individual cases before the diagnostic melanosome is observed.

The standard classification of melanosomes recognizes four stages of melanosome development [25]. The first stage contains no melanin. The second stage (melanosome Stage II or premelanosome) contains the characteristic patterned membranous structure that has been thought of as a folded membrane, a collection of filaments, a concentric sheet, or a helical tubular structure. Deposition of melanin on the tubular structure signals the next stage (melanosome Stage III, partially melanized melanosome, or premelanosome). The final product is a densely melanized granule without discernible internal structure (melanosome Stage IV, mature melanosome).

It is now believed that, in rare instances, other tumors composed of cells of neural crest origin, such as Schwann cells and perhaps certain neuroendo-

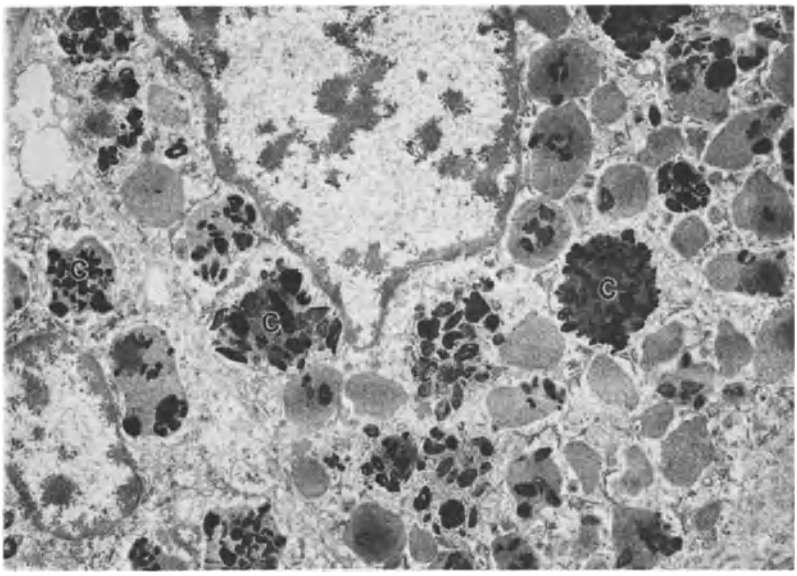

Figure 7. Macrophage (melanophage) containing numerous intracytoplasmic compound melanosomes (C); (×12,000). Courtesy of Dr. S. Bhuta, UCLA.

crine cells, are also capable of producing melanosomes. The principal feature that distinguishes the melanocyte is that it contains discrete or solitary melanosomes in various stages of development and melanization. Other distinguishing features include: (1) melanocytes do not form desmosomes or other types of cell junctions with one another or with adjacent cells. (2) The cytoplasmic matrix of the melanocyte is much lighter (less electron-dense) than that of the keratinocyte. (3) Tonofilaments are absent and intracytoplasmic filaments of other types are sparse. (4) The Golgi complex is fairly well developed [26].

Melanosomes may occur as discrete solitary organelles or they may occur in groups surrounded by a single membrane (known as a compound melanosome or a melanosome complex; Fig. 7). Compound melanosomes are usually seen in melanophages and are only rarely seen in melanocytes. Like the normal melanocytes, the neoplastic melanocyte is characterized by solitary melanosomes in various stages of development. Compound melanosomes are only very rarely seen and then they are quite small. Since both solitary and compound melanosomes may be found in keratinocytes, it is not surprising that melanosomes may be found in some squamous and basal cell carcinomas. However, the presence of melanosomes in these tumor cells does not usually present a diagnostic problem, as the presence of tonofilaments and desmosomes clearly distinguishes these tumors from melanomas.

The greatest diagnostic pitfalls occur because of neoplasm-associated morphological alterations in melanosomes and the resulting confusion with other similarly shaped organelles. The unequivocal identification of a melanosome depends on the observation of its characteristic membranous internal structure. Lyosomes are organelles that may be confused with melanosomes, and myelinosomes (lysosomes containing myelinoid membranes) are particularly apt to be a source of confusion. Granules from mast cells and basophils may also be mistaken as melanosomes; however, the internal structure of melanosomes is quite different from that of the granules of mast cells and the basophilic leukocytes. Close scrutiny should therefore resolve any possible confusion.

Finally, certain profiles of the rod-shaped microtubulated body (Weibel-Palade body), the specific organelle of vascular endothelia, may resemble melanosomes. In practice, this organelle rarely provides a diagnostic dilemma in the differential diagnosis of malignant melanoma. Microtubules showing various configurations have been found in rough endoplasmic reticulum. The so-called straight or rod-like microtubules found in rough endoplasmic reticulum may provide additional evidence that a tumor is a malignant melanoma. A few randomly scattered microtubules may be present, or parallel microtubules may be sufficiently well ordered to qualify as having a 'crystalloid' or 'crystalline' arrangement may be found. In some instances, the microtubules are set in a medium-density background, while in other cases they are set in a light matrix. Although rod-like microtubules have been identified in the rough endoplasmic reticulum of different kinds of cells, the largest number have been observed in malignant melanomas. They have even been detected in tumors where no melanosomes were noted. In these instances, these structures may suggest, although not prove, the diagnosis of malignant melanoma.

The identification of premelanosomes and rod-shaped microtubules is thus generally a reliable adjunctive means of diagnosing tumors of melanocytic origin.

3. Histology of variants of primary melanoma

While all melanomas are unified by their common cell of origin, they may be subdivided on the basis of their clinical appearance, growth kinetics, and histology. These subdivisions are of three types. There are the histogenetic subdivisions of the commonly occurring types of cutaneous melanoma: a majority of melanomas having a radial growth phase (superficial spreading melanoma and lentiginous melanomas) and a minority lacking a radial growth phase (nodular melanoma; Fig. 8). There are unusual variants of melanoma that include desmoplastic/neurotropic melanoma and myxoid melanoma, and the melanomas that resemble benign melanocytic nevi (minimal deviation melanoma) or nonmelanocytic lesions. Finally there are

the melanomas that arise from melanocytes in unusual locations or from melanocyte-derived or -related cells. It is important to be aware that of these melanoma variants, as some are major diagnostic challenges to the clinician and pathologist alike, some may require slightly different surgical approaches and others pursue a clinical course that is quite different from that of the majority of melanomas.

3.1. Histogenetic subtypes of commonly occurring types of melanoma [27–30]

All invasive melanomas exhibit a tumor mass or nodule that extends from the epidermo-dermal junction into the underlying dermis, and on the basis of this invasive component alone it is not possible to tell one type of melanoma from another.

In a minority of melanomas the invasive component is the tumor, and the tumor appears on clinical inspection and microscopy to have arisen from normal skin (*nodular melanoma or melanoma without an adjacent radial growth phase*; Fig. 8). Such tumors appear to develop quite rapidly, and the history will often be of a pigmented tumor developing from clinically normal skin over a period of 9–18 months.

The remaining cutaneous melanomas develop their invasive component from an antecedent or precursor lesion that is recognizable both clinically and microscopically. These lesions appear to follow a similar evolutionary path. Following malignant transformation, a patch of malignant melanoma in situ develops. This lesion consists of a proliferation of transformed melanocytes that are present singly and in colonies (nests), with single

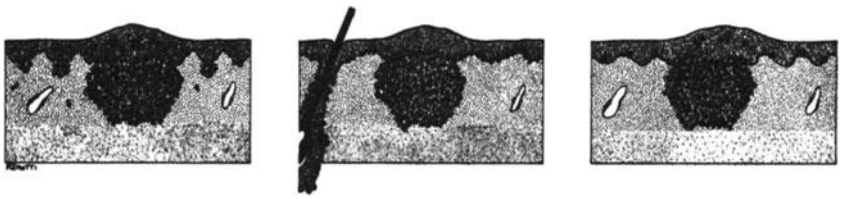

Figure 8. Subtypes of melanoma, based on the presence or absence of a radial growth component and the histology of the radial growth component. The left panel shows invasive melanoma arising against a radial growth component in which abnormal melanocytes are present singly and in colonies in the suprabasal epidermis and at the epidermo-dermal junction (Pagetoid or superficial spreading melanoma). The middle panel shows invasive melanoma arising against a radial growth component in which abnormal melanocytes are present singly and in colonies, predominantly at the epidermo-dermal junction of the surface epidermis in a comparable position in the skin appendages (lentigo maligna melanoma). A similar radial growth component is seen in acral lentiginous melanoma and mucosal lentiginous melanoma. The right panel shows invasive melanoma arising against a normal epidermis, with no evidence of a radial growth component (nodular melanoma, melanoma without a radial growth component). Note that suprabasal melanocytes in the epidermis over the invasive tumor does *not* make this a pagefoid melanoma.

melanocytes usually predominating over nests, at the epidermo-dermal junction of the interfollicular epidermis and in the skin appendages (*melanoma with a radial growth phase*). In the majority of such tumors, the atypical melanocytes in the radial growth phase are present both at the junction and within the epidermis, in the latter site presenting a pattern that resembles Paget's disease (pagetoid penetration of the epidermis; Fig. 2). Such melanomas are classified as *superficial spreading melanomas*. In patients with this type of melanoma, a history will often be obtained that a changing pigmented lesion had been present for a period of up to 5 years. This is the type of melanoma that most frequently develops in association with dysplastic nevi. The other major pattern of radial growth phase is where the proliferation of atypical melanocytes is predominantly confined to the junction between epithelium and the underlying connective tissues, with involvement of adnexal structures (Fig. 8). When this occurs on sun-exposed skin (face and scalp, backs of hands, etc.), this is classified as *lentigo maligna melanoma*. A similar type of radial growth phase associated with tumors on acral sites (sole of feet and palms of hands) classifies the tumor as an *acral lentiginous melanoma*, while on a mucosal sites such as the conjunctiva, vagina, or anus, the tumor is described as a *mucosal lentiginous melanoma*. Lentiginous melanomas generally evolve at a comparatively slow rate, and in the case of lentigo maligna melanoma a progressively (if subtly) altering flat pigmented lesion may have been present for up to 15 years. It has to be admitted that it is sometimes very difficult to place an individual tumor in one of these categories, especially in the absence of clinical information, and it is probably most appropriate, in the face of interpretative difficulty, to describe the lesion as *malignant melanoma with a radial growth phase of unclassifiable type*.

We consider that the clinical presentation and evolution of these different forms of melanoma is sufficiently different to warrant their being regarded as separate. We do not argue that the prognosis for a patient with a melanoma of identical thickness and depth of invasion is broadly similar, regardless of the histogenetic type of the tumor. We do, however, believe that the natural history of the melanoma variants is quite different. Put in the simplest terms, starting at the point of melanocytic transformation, it takes a great deal longer for a lentigo maligna melanoma to reach Clark level III and 2.0 mm than it does a nodular melanoma.

A further reason for subtyping melanomas is the need to excise completely any radial growth phase that is present, as to leave any melanoma in situ in place virtually dooms the patient to at least local recurrence.

3.2. Unusual variants of melanoma

3.2.1. Desmoplastic melanomas. These are melanomas comprised of spindle-shaped tumor cells embedded in an abundant collagenous stroma [7]. It appears that the melanoma cells secrete growth factors that stimulate local fibroblasts, which in turn produce the collagen of the exuberant stroma.

Such tumors may present considerable diagnostic difficulty clinically for the microscopist. Clinically, being hypomelanotic or amelanotic, they may appear as an area of vitiligo or as a scar. They may also present as nodal metastases, and the primary may be identified only when the nature of the tumor in the lymph node is appreciated. At the level of light microscopy, they are to be separated from a range of spindle-celled processes that includes scar tissue, dermatofibroma, atypical fibroxanthoma, spindle cell squamous carcinoma, fibrosarcoma, and neurofibrosarcoma. The process of diagnosis at the microscopic level is facilitated by immunohistochemistry, though the cells of some desmoplastic melanomas do not react with standard reagents, such as HMB45, though most are S-100 protein positive. In this situation some assistance is provided by the observation that most desmoplastic melanomas arise from lentigo maligna melanoma or from acral lentiginous melanoma. Careful sampling of the tumor and its surrounding skin will often provide evidence of a radial growth phase that will clinch the diagnosis. Desmoplastic melanomas often spread to ensheath and infiltrate the cutaneous nerves (*neurotropism*; Fig. 5). Identification of this process is of the highest importance, as failure to excise these tumor extensions makes it likely that the tumor will regrow from this residual tumor.

3.2.2. Myxoid melanoma. These are melanomas in which the tumor cells, which may be epithelioid or spindle shaped, are embedded in a myxoid stroma that is rich in hyaluronic acid [31]. Myxoid change is most commonly seen in metastatic melanomas, but we have occasionally seen such an alteration in primary melanoma. The tumors are seldom diagnosed correctly at the first attempt and are to be separated from other more common types of myxoid tumor, such as myxoid liposarcoma and myxoid chondrosarcoma. The thoughtful application of immunohistochemistry can be of great assistance in this situation.

3.2.3. Melanomas that resemble benign melanocytic tumors. A proportion of melanomas that is fortunately relatively small can, to some degree, resemble a benign melanocytic nevus, Spitz nevus, or Reed nevus [4,6]. The best protection against underdiagnosis of such lesions is to be continually aware of the potential pitfall. Whenever a changing nevus is encountered, especially in an individual beyond the age when new nevi are expected to develop, ask the question, is there any possibility that this could be a melanoma? The criteria that we utilize to diagnose such lesions (and it may be necessary to examine multiple levels and sections to identify them) include active junctional activity and pagetoid penetration of the epidermis, cellular pleomorphism in the dermal component, continued melanogenesis in the deep part of the tumor, mitotic activity in the deep tumor cells, atypical mitoses, and a lymphohistiocytic infiltrate in response to the tumor. Such tumors are undoubtedly malignant and may metastasize to the regional lymph nodes. Systemic metastases may be less frequent than with conven-

tional melanomas. We consider that these are relatively well-differentiated melanomas. Other authors have described these as *minimal deviation melanomas* [32], an entirely logical description that, for no very apparent reason, has generated some mystification and has found acceptance and competent application very slowly.

3.2.4. Amelanotic melanoma. Primary amelanotic melanomas do occur, though few are truly amelanotic, especially if the inspection includes assessment of any radial growth phase that is present. Truly amelanotic metastases are more common. The ultrastructural demonstration of premelanosomes or melanosomes will further reduce the proportion of 'amelanotic' tumors.

The incidence of these lesions is difficult to determine, as most series do not define criteria for consideration of a melanoma as amelanotic. The incidence varies depending on whether only primary tumors are considered or whether metastases are included. The incidence of amelanotic melanomas varies from 1.9% to 8.1% in major series. Of 2881 melanoma patients seen at the University of California, Los Angeles, 50 (1.8%) had either an amelanotic primary tumor or amelanotic metastases. Twenty-nine patients presented with an amelanotic primary, and 21 had amelanotic lymph node metastases from a melanotic primary. There was a total absence of pigment in both the primary and in its metastases in only three patients [33].

Diligent examination of thin, adequately fixed HE-stained sections reveals melanin in most cases. To establish the diagnosis, it is necessary to demonstrate fine granules of intracytoplasmic melanin (individual melanosomes) in tumor cells and to demonstrate the nature of the granules by a specific melanin stain, removal of the pigment by bleach, and the absence of positive reactions to iron or lipid stains. In fact the great majority of amelanotic melanomas are readily identified by showing their reactivity with antibodies to S-100 protein, HMB-45, and/or NKI-C3, and the absence of reaction to antibodies to common leukocyte antigen and cytokeratin (see above).

The prognostic significance of absent or deficient melanin synthesis in a melanoma is the subject of considerable controversy. Some reports suggest that absent pigment production is associated with poor survival, but most studies have found that the pigment content does not affect prognosis [33].

3.2.5. Metastatic melanoma without an identified cutaneous primary site. A small proportion of melanoma patients present with lymph nodal or (more rarely) visceral metastases of melanoma in the absence of a demonstrable primary tumor. Most such patients will have either have had an undiagnosed melanoma excised or destroyed, or spontaneous regression of a primary melanoma [34]. Vital information can be obtained by a careful and detailed clinical history, taking note of any skin lesions previously removed or that have undergone spontaneous regression. The patient's skin should also be examined for old scars or areas of depigmentation (the latter process is

facilitated by the use of a source of ultraviolet light, such as a Wood's lamp). This approach will reveal the site of a probable regressed/treated primary melanoma in most but not all patients. An interesting recent suggestion is that some melanomas may be primary in the lymph nodes [35], possibly arising from the nevocyte aggregates that occur in the capsule of about 6% of nodes [36].

3.2.6. Melanomas that arise primarily in the dermis. The overwhelming majority of primary cutaneous melanomas arise from melanocytes at the dermo-epidermal junction. Dermal primary melanomas are thus infrequent, the bulk of malignant melanocytic tumors in the dermis being cutaneous metastases. Primary dermal melanomas arise either from the malignant transformation of dermal nevocytes of (usually congenital) nevocytic nevi or from conventional or cellular blue nevi. Transformation of dermal nevocytes is frequent in the congenital nevi of young children that undergo malignant degeneration, but is highly unusual in the malignant transformation of congenital nevi in adults.

Malignant transformation of conventional blue nevi [37,38] and of cellular blue nevi [39] is well recorded but rare.

3.2.7. Melanomas of the soft tissues. The most important lesion of this type is the malignant melanoma of the soft parts [40], formerly known as the *clear cell sarcoma of the tendon sheath.* Such tumors arise mainly on the extremities as subcutaneous masses in the area of tendons and aponeuroses. The cells are spindle to round in shape and tend to form nests and fascicles in which multinucleated forms are often visible. Melanin synthesis is limited and melanosomes are scarce. Mitoses are usually detectable but infrequent. The tumor cells are PAS-positive, silver (Fontana)-positive, and contain S-100 protein. The tumors must be separated from spindle-cell melanoma, synovial sarcoma, epithelioid Schwannoma, and metastatic melanoma or clear-cell renal carcinoma.

Occasional malignant Schwannomas may be (weakly) melanogenic and contain sparse melanosomes. Separation requires careful considerations of the clinical context. It may be difficult to distinguish such tumors from spindle cell melanoma and impossible in the absence of immunohistochemistry with monoclonal to the subunits of S-100 protein (see above).

3.2.8. Malignant melanomas that resemble nonmelanocytic lesions. This problem should not arise in melanogenic primary melanomas where the epidermis is at least partially intact, preserving junctional growth nests or other evidence of a radial growth phase.

Keratinocyte-derived lesions, such as squamous papilloma, seborrheic keratosis, actinic keratosis, basal cell carcinoma, or squamous cell carcinoma, may appear pigmented on clinical and microscopic examination, but in well-oriented, vertically cut sections these should be easily recognizable as

nonmelanocytic. Such lesions may show an incidental increase in melanocyte numbers as well as in melanogenesis. The pigment cells are never extensively pleomorphic, are singly dispersed, and show no tendency to nest formation or invasion.

Ulcerated tumors or tumors excised without the overlying epidermis can be troublesome, especially where the cells are spindle shaped. The differential diagnosis of spindle cell tumors includes lesions as diverse as leiomyoma, dermatofibroma, neurofibroma [41,42], sclerosing angioma (dermatofibroma, histiocytoma), fibrous histiocytoma, Schwannoma (especially the pigmented variety) [43], atypical fibroxanthoma, and 'Triton' tumors. Epithelioid tumors have to be separated from clear cell sarcoma of tendons and aponeuroses [40], breast cancer metastatic to the skin [44], Merkel cell tumors, and cutaneous deposits of lymphoma. This may be difficult, especially in poorly fixed, stained, or badly oriented material. Special stains, ultrastructural studies, and special techniques, such as staining with anti-S-100 protein, are of value.

3.3. Microscopy and prognostication

3.3.1. Assessment of the likelihood that a patient will develop metastases based on evaluation of characteristics of the primary tumor. The main parameters that are useful in assessment of prognosis are those that relate to the size of a primary melanoma. Tumor size correlates broadly with the time that the tumor has been present and its capacity to grow progressively. Also, the longer a tumor is present the more probable it is that it will develop the capacity to metastasize.

The association between the size of a primary melanoma and the likelihood of metastases has been known for many years. Tumor size was initially evaluated by assessing whether the tumor was superficial or deep [45], by examining the sectional profile of the primary tumor (flat vs. convex vs. polypoid) [46], or evaluating whether the deepest invasive tumor cells lay above or below the sweat glands [4,47].

The assessment of primary tumor size was made more precise by Clark [48], who related the depth of invasion by melanoma cells to standard landmarks of the skin: the dermo-epidermal junction, papillary dermis, papillary-reticular interface, the reticular dermis, and the subcutaneous fat (Fig. 9). This remains a practicable approach, with patients with tumors of more superficial Clark levels (I–II) seldom developing metastases relative to those with deeper tumors (III–V). The technique has value in assessing prognosis, particularly in its capacity to separate tumors above and below the dermo-epidermal junction or the papillary-reticular dermal interface. There are practical problems in applying this approach [49]. The papillary dermis and the reticular dermis are difficult to identify or separate in some areas of the body, such as the acral areas, the papillary dermis being poorly developed in these sites. The reference landmarks may be obscured by a

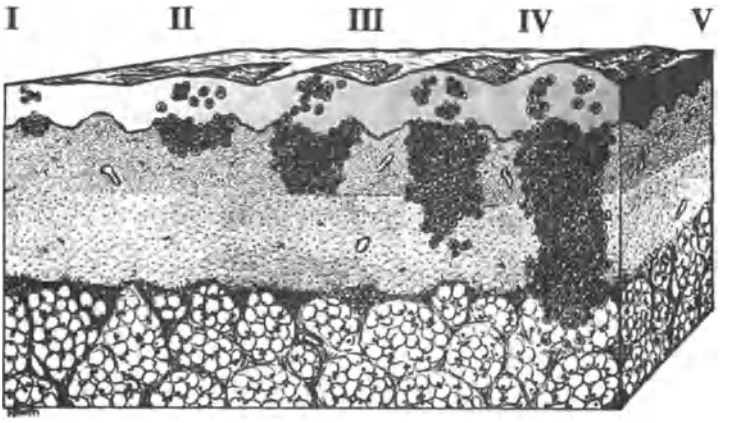

Figure 9. Levels of invasion of the skin. After Clark [48], with permission. See the example attached and the text.

dense lymphoid infiltrate, 'regressive' fibrosis, or by a preexisting nevus. Where there are many hair follicles, it can be difficult to separate direct invasion from extension down the sheath of a hair follicle. This may easily lead to overestimation of the depth of invasion.

Clark's approach has been partly replaced by the micrometer-measured assessment of thickness described by Breslow [50]. This remains the most useful single microscopic feature that relates to prognosis. Tumor thickness is quantified to the nearest tenth of a millimeter with an ocular micrometer (Fig. 10), measuring from the top of the granular layer of the overlying epidermis or the base of a tumor ulcer, to the deepest *contiguous* invasive melanoma cell. There is a direct correlation between tumor thickness and the probability of the development of metastases. Thickness measurements must not include subjacent noncontiguous satellite foci of tumor, areas of vascular invasion, or tumor sheathing epidermal appendages. The technique must not be uncritically applied, for example, to tumors where the overlying epidermis is substantially thickened (acral sites or reactive hyperplasia), as this will overestimate true tumor thickness and may bring about serious overtreatment. In the presence of extensive 'regressive' fibrosis, measurement is made to the deepest certainly identifiable tumor cell and the measurement must be qualified by the statement that regression is present. It is essential not to misidentify melanin-containing macrophages (melanophages) as tumor cells.

Major problems in applying Breslow's technique may be encountered when a melanoma coexists with and blends with cells showing nevocytic morphology. There is no real problem when a melanoma abuts a preexisting nevus, the Breslow measurement being made to the deepest identifiable melanoma cell. The situation is more difficult when melanoma cells blend with a population of nevocytoid cells, as it is necessary to separate a

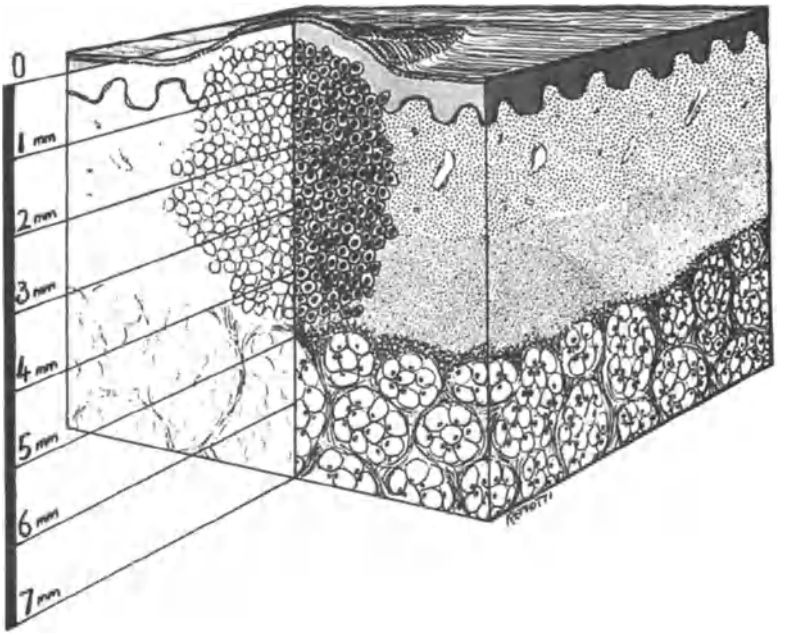

Figure 10. Tumor thickness measured by micrometer (see text). After Breslow [50], with permission.

melanoma showing nevocytic differentiation in its deeper portion from melanoma infiltrating a preexisting nevus. Assistance can be obtained from an examination of the nevocytic portion of the tumor under polarized light or in sections stained for reticulin, the reticulin framework generally being lighter and less prominent around nevocytically differentiated melanoma cells than around true nevocytes. It is also possible to stain the tumor for the melanoma-associated epitope HMB45 that is present in most melanoma cells, including those that are nevocytically differentiated and absent from true intradermal nevocytes. If after these approaches have been applied doubt persists, the surgeon should be provided with a measurement that includes all melanocyte-derived cells in the dermis, but should be advised of the nature of the dilemma.

Most errors in applying Breslow's technique result from simple mathematical errors in converting the micrometer measurement to millimeters or from attempting to use a microscope that has not been calibrated for use with the micrometer in use.

Other histologic characteristics of primary melanoma correlate with prognosis, but none have the power of the Breslow or Clark approaches. The most relevant of these observations include the micrometer-measured width of ulceration [51], the frequency of mitotic figures [4,46,52], the

presence of tumor within blood or lymphatic vessels [4,46,53], the presence of 'microsatellites,' and the presence and distribution of lymphohistiocytic infiltrates in the peritumoral area and within the area of the tumor [4,10,47,54].

A proportion of melanomas show foci of fibrosis, lymphohistiocytic infiltrate, and increased vascularity, which are generally regarded as evidence of regression. The significance of these areas is twofold: With development and contraction of the fibrous tissue, the true depth/thickness of the primary tumor may be underestimated [55] and lesions with extensive regressive change (>70%) appear to have an unfavorable prognosis [56].

Attempts have been made to increase the accuracy of assessment of probable clinical outcome by combining clinical and pathological features into a prognostic score sheet or formula. We initiated this approach in 1968 [57] and its utility was confirmed by MacKie et al. [58]. Other similar schemes have been presented [59], the most recent being that by Clark et al. [60]. Multifactorial approaches do increase the accuracy of prognostication but have not yet gained wide acceptance. The increase in prognostic accuracy has generally been insufficiently large to justify the extra labor involved. There remains an urgent need for a system that, in return for a modest increment in effort, would allow the accurate assessment of prognosis *on an individual patient basis*. For the present, most centers continue to apply the Breslow and/or original Clark approaches.

4. Precursor lesions for melanoma

Much of the improvement in the outlook for the patient with melanoma has come from the development of the capacity to identify melanomas early in their development at a time when local excision is curative. No one would dispute that the prognosis for the patient with a thin, superficially invasive primary cutaneous is better than that of individuals with deeper, thicker lesions. Similarly the patient with melanoma in situ is probably not at risk for the development of metastases, while patients with invasive lesions certainly are. It is now considered that, just as melanoma in situ can be recognized clinically and microscopically, there are a series of identifiable lesions that, while not in themselves malignant, represent stations on the evolutionary pathway that may eventually lead to fully developed, potentially metastatic, and thus potentially lethal, cutaneous melanoma. Simple excision of such lesions clearly prevents their further evolution, though patients who develop such lesions are likely subsequently to develop other similar lesions and should be advised on the extent of their solar exposure and examined regularly.

Broadly the identifiable precursor lesions of melanoma are pigmented macules and papules that are irregular in their appearance and growth kinetics. They tend to be larger than conventional acquired nevi, have a more irregular border, and show variegations in pigmentation. In contrast to

conventional moles that, after they achieve their full size, are essentially unchanging (other than undergoing slow atrophy with age), melanoma precursor lesions grow in area or height and change their shape.

Recognized precursors for melanoma include congenital nevi [61], acquired nevi [62], particularly the atypical 'dysplastic nevi' [63,64] that show melanocytic dysplasia (a linear and nested increase in atypical melanocytes in the basal epidermis, with a 'windblown' morphology of the nests that may link adjacent rete pegs), and secondary alterations in the epidermis (lamellar fibrosis, concentric eosinophilic fibrosis, neovascularization, and lymphohistiocytic accumulation) and lentigo maligna [65].

It is of the utmost importance that all physicians are aware of the clinical appearance of these lesions so that they may be excised early and before they have evolved to a stage where metastases may occur.

5. Pathology and prognosis in node-spread melanoma

The outlook for patients with node-spread melanoma (Stage II) has improved since the 1950s and 1960s, when 80% of patients died of melanoma. Now, about 50% of Stage II melanoma patients are tumor-free 5 years after therapeutic lymph node dissection (TLND), and most such individuals die of causes other than melanoma [66]. There remains a need to predict outcome for the individual patient at the time of node dissection.

Prognostic factors include time from primary excision to node metastases, thickness and width of ulceration of the primary, histologic subtypes of primary melanoma, amount of melanoma in the nodes (e.g., clinically palpable vs. histologically detectable tumor), number of tumor-containing nodes, and extracapsular extension of tumor [66–69].

The number of melanoma-containing nodes correlates with outcome: Retrospectively, 47% of patients with up to three tumor-positive nodes survived 5 years, whereas only 21% with tumor in four or more nodes survived 5 years. Prospectively this approach accurately predicted survival (85% correct) but underestimated deaths from melanoma (40% correct). The number of tumor-containing nodes imprecisely indicates tumor burden, a patient with three tumor-replaced nodes being assessed as having the same prognosis as one with three nodes containing tumor microfoci.

Measurement of tumor volume relative to the volume of the affected group of nodes would likely be the best predictor of subsequent outcome, but such measurements are not really practical outside the research laboratory. We therefore have used an ocular micrometer to measure the aggregate diameter of tumor in the nodes (ATD; micrometer-measured tumor thickness is a surrogate for tumor volume in primary melanoma) to supplement other indicators of clinical outcome (Fig. 11) [70].

When ATD was less than 15% of total lymph node diameter, patients seldom had melanoma recur after lymphadenectomy (2; 13–15%). Indi-

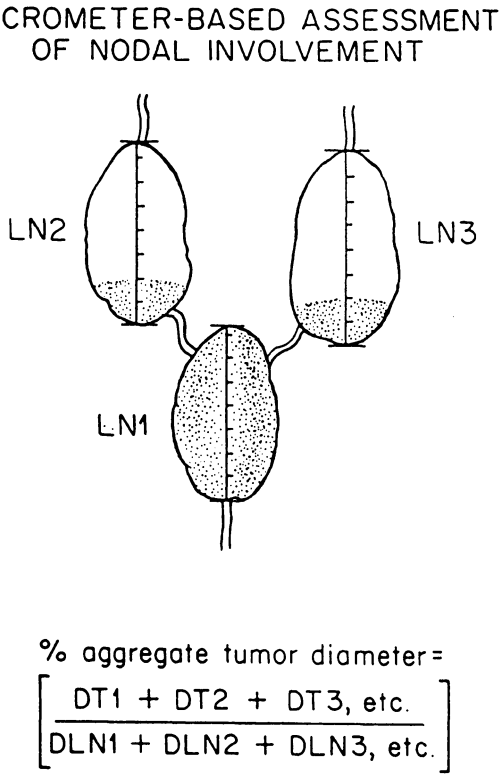

Figure 11. Technique for assessing the proportional maximum diameter of melanoma in a lymph node group. Maximum tumor and nodal diameter for each node are measured using a micrometer. The data from each node are summed, and the aggregate tumor diameter is expressed as a percentage of aggregate nodal diameter.

viduals in whom ATD was greater than 15% were more likely to have recurrences (10; 15–67%). Assessment of tumor diameter accurately predicted outcome in 21 of 28 patients (75%). We examined the predictive power of combining number of nodes containing tumor and aggregate tumor diameter. Tumor in three or fewer nodes and an ATD less than 15% accurately predicted favorable outcome for 12 of 13 patients (92%). Fatal progression was correctly predicted by four or more positive nodes and ATD greater than 15% for 12 of 15 individuals (80%). The combination of number of tumor-containing nodes and tumor diameter accurately predicted outcome for male and female patients, and for patients who had nodes removed from the groin, axilla, and cervical areas.

To assess prognosis accurately for Stage II melanoma patients, for example, for stratification in clinical trials, assessment of the number of tumor-positive nodes should be supplemented by micrometer-measured

aggregate tumor diameter. Immunohistologic approaches to detect occult tumor may further enhance prognostic accuracy.

6. Nuclear measurements of metastatic melanoma cells and prognosis

Nuclear DNA ploidy analysis using flow and static DNA cytometry define nuclear aberrations, which may provide additional prognostic information. Patients with aneuploid Stage I melanoma have a lower survival than those with diploid tumors [71,72]. We recently studied 22 patients post therapeutic lymphadenectomy, using static DNA cytometry to characterize nuclear DNA abnormalities in lymph nodal metastases of melanoma, and the biologic significance of such abnormalities [73]. Nuclear characteristics that correlated with survival included ploidy, number of clones present, and the coefficient of variation of DNA content. Eight of 15 patients with two or more tumor cell clones died within 14 months, whereas all seven patients with monoclonal metastases survived more than 14 months. Other have noticed that patients with polyclonal tumors have an unfavorable prognosis. The use of nuclear DNA measurement techniques seems likely to become more widespread.

7. Assessment of the dissection specimen to determine the completeness of a lymph node dissection

Recurrence of melanoma after lymphadenectomy in the area of a lymph node dissection is usually a grave occurrence, rapidly followed by death. Regrowth often occurs from tumor-containing nodes not removed at initial lymphadenectomy. There is, therefore, a need to assess the completeness of a node dissection. This require meticulous examination of the specimen to identify and count all nodes present. From a study of 72 dissections from 52 melanoma patients, the mean number of nodes in the axilla is 19 and in inguinal dissections 17. When a full lymphadenectomy is undertaken, the specimen should contain a number of nodes close to the above. This requires the surgeon to dissect meticulously all nodes in the area, and the examining pathologist equally meticulously to examine the excised specimen and to identify all nodes in it. What is the correct action when the number of nodes identified in a lymphadenectomy specimen is substantially below that expected? We need to determine the frequency with which local regrowth follows such an observation to decide whether to undertake immediate reoperation to complete the lymphadenectomy, to administer local radiation, or whether such patients can reasonably be managed on a 'wait and see' basis.

Assessment of the tumor status of lymph nodes by inspection and palpation is an ineffective approach to the identification of patients with early spread of melanoma. In a study of nodes removed by elective node

dissection, up to 15% of patients were found, by microscopy of hematoxylin and eosin stained slides, to have small deposits of melanoma in nodes evaluated as tumor free on clinical assessment. Immunohistochemistry of nodes from the 85% of patients who had been evaluated as tumor free by clinical examination and scrutiny of H&E stained slides revealed an additional 14% of patients to have nodes that contained small numbers of 'occult' tumor cells [23]. Thus, up to 29% of melanoma patients regarded as having localized disease by standard evaluation techniques actually had metastases in the regional nodes. There may, therefore, be a need to utilize immunohistochemistry in evaluating lymph node specimens.

Because we see many patients who have high-risk primary melanoma, and because of concerns about the 'blind' application of Elective lymph node dissection (ELND) to all such patients, we have recently developed an alternative management for such patients. We now routinely use the techniques of sentinel node localization by blue dye and intraoperative evaluation of nodal tumor status to provide a logical basis on which to determine those patients most likely to derive real benefit from nodal dissection.

8. Pathology of the regional lymph nodes of patients with high-risk primary melanoma, including those managed by dye-directed selective lymphadenectomy

The management of patients with high-risk primary melanoma by dye-directed selective lymphadenectomy developed from two advances: the capacity to identify very small numbers of tumor cells in tissues, including lymph nodes, and the ability to identify in vivo the lymph nodes nearest to a primary melanoma on the relevant lymphatic drainage pathway (sentinel nodes). We initially reported S-100 protein as a practical marker for benign and malignant cells of melanocytic lineage [13] and then reported that in Stage II melanoma, 40% of ostensibly tumor-free nodes actually contain a few tumor cells detectable by antibodies to S-100 protein [22]. In nodes excised electively from patients with high-risk primary melanoma, antibodies to S-100 protein and the melanoma-associated epitopes, NKI/C3 and HMB 45/50, showed that 14% of patients had tumor cells in one or more nodes [23] (Fig. 12). This is in addition to the 15% of patients who, despite negative clinical evaluation, had nodal tumor on conventional pathological assessment. To permit intraoperative evaluation of nodes, we developed a rapid immunohistochemical technique that allows accurate assessment of a node in under 30 minutes [74].

Techniques were developed to identify the nodes nearest to primary melanoma on the relevant lymphatic pathway, the nodes most likely to contain metastases. We examined lymphatic drainage in young cats by injecting marker dye intradermally into the thighs and lower abdomen. The inguinal nodes were exposed, and the passage of dye along the lymphatics

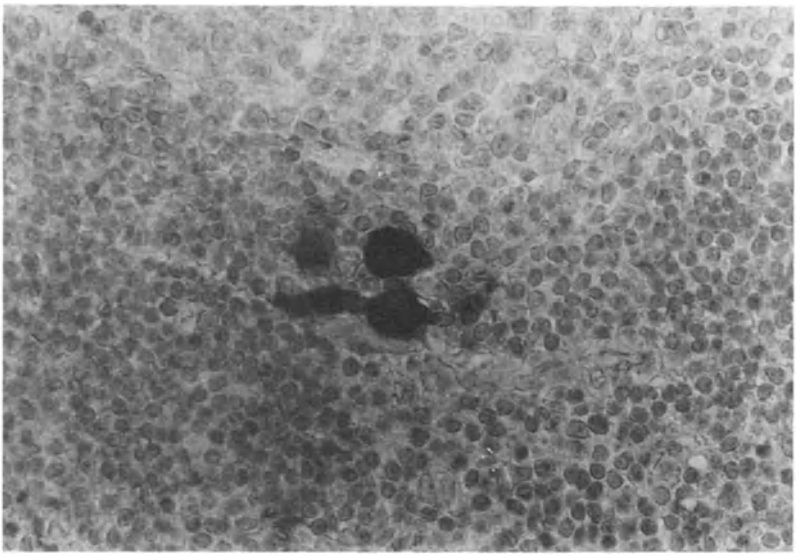

Figure 12. Occult melanoma cells in a lymph node regional to melanoma. Peroxidase-antiperoxidase-aminoethylcarbazole technique. Polyclonal antibody to S-100 proten (×500).

was followed to the nodes, which were observed for blue coloration. Routes of lymphatic drainage were constant and essentially identical from cat to cat [75].

The studies were extended to melanoma patients. Marker dye was injected intradermally adjacent to the primary melanoma or at the site of the excision biopsy of a melanoma. The regional nodes were surgically exposed, and afferent lymphatics were inspected for the passage of marker dye. Lymph nodes that became blue were excised with a 2 cm margin of surrounding tissue and were immediately examined by frozen section and rapid immunohistochemistry to identify any melanoma cells present. While pathological evaluation was in progress, the area of the primary tumor was widely excised. *If sentinel lymph nodes were found to contain tumor, a full lymphadenectomy was performed* [74].

At least one sentinel node was identified in 81% of 243 patients managed in this manner. The success rate varied slightly with the site of the nodes (groin, 89%; axilla, 78%) and as surgeons gained experience (61–97%). Most patients had one sentinel node, but 20% had two, and 8% had three or more. A sentinel node was identified in 194 lymphadenectomy specimens, and 40 of those sentinel nodes (21%) contained metastatic melanoma. Tumor was identified in H&E stained sections in 23 instances (57%) and exclusively by immunohistochemistry in 17 (43%). More recently, with experience the proportion of cases identified in H&E-stained tissues has increased. Melanoma was identified in 62 of 3332 lymph nodes (2%). The metastases

were exclusive to the sentinel lymph nodes in 47 patients (76%), but in 13 patients (21%) the metastases also occurred in nonsentinel nodes that lay within 2 cm of the sentinel nodes. In the early part of the study, when all patients were subjected to full lymphadenectomy, regardless of the outcome of the sentinel node evaluation, metastases were found exclusively in non-sentinel nodes in only two patients (1%). This has not been encountered in our most recent 200 patients, and we consider these two patients to be exceptional, reflecting our initial ignorance of the kinetics of dye flow. *We have never encountered true skip metastases.*

How accurately can small numbers of melanoma cells be identified intraoperatively? Exact concordance between evaluation of the intraoperative and permanent preparations was achieved in 220 of 227 patients (97%). In 36 patients, tumor cells were identified intraoperatively and were confirmed in the permanent preparations. No false-positive report was issued on a frozen section. In 191 patients, tumor cells were identified neither intra-operatively nor in the final material. In seven patients, tumor was not identified intraoperatively but was present in the final preparations. This represents a false-negative rate of 3%, a figure low in the range of published false negatives for routine frozen sections (3–5%). Such errors usually reflected an insufficient initial sampling of the nodal tissues. An acceptable alternative would be to evaluate the sentinel node intraoperatively by the conventional H&E approach, performing immunohistochemistry only on the permanent material. By this approach a small number of patients would require a second operation to complete the lymphadenectomy.

Identification of single tumor cells in nodal tissues can be difficult; a difficulty reduced, but not abolished, by experience. Technically excellent, thin, well-stained sections are essential. Melanoma cells in frozen sections may stain less intensely with antibodies to S-100 protein than do melanoma cells in formalin-fixed material, and not all melanomas express the epitopes detected by the melanoma-directed monoclonal antibodies NKI/C3 and HMB 45/50. Difficulties in intraoperative interpretation may thus be compounded by the weak or absent staining of melanoma cells in frozen material.

Care must be taken that the immunoreactive cells identified in the nodes are in fact melanoma cells. A variety of cells in the nodes will react with the antibodies employed, and factors such as the position of the cells in the nodes and the size and cytologic features of the cells must be taken into account. Melanoma cells are generally much larger than lymphocytes and macrophages, and have a relatively high nucleo-cytoplasmic ratio. In the early stages of invasion, at the point when they are truly occult, they usually lie in the peripheral sinus.

Nodal nevus cells can stain with any or all of the antibodies in use but are small, have scanty cytoplasm, and are almost located in the nodal capsule. Paracortical interdigitating cells stain with S-100 protein, but not with HMB 45 or NKI/C3. They lie in the interfollicular areas and in the paracortex, they are usually more or less dendritic, and they have reniform nuclei. A variety of cells in the sinuses may stain positively. These are NKI/C3+

macrophages and S-100 protein-positive veiled cells (lymphoid dendritic cells in a nondendritic transport mode) and some lymphocytes. These are smaller than melanoma cells and tend to stain less intensively with S-100. Very occasionally a nerve will traverse a node, and in that case the Schwann cells will stain with S-100 protein.

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5. The prevention of cutaneous malignant melanoma: High-risk groups, chemoprevention, education, and screening

Mark H. Greene

1. Introduction

The ultimate strategy in the control of any disease is prevention. Nowhere in medicine is this more true than in oncology, as the physical, emotional, social, and economic morbidity consequent upon the natural history of neoplastic disease and its treatment (even when successful) is truly enormous. The ability to prevent a given disease has typically lagged far behind our ability to treat that disease. Historically, physicians have been accustomed to focusing on the treatment of established disease, an enterprise that can be undertaken without any knowledge of the disorder's etiology and pathogenesis. In addition, prevention efforts shift the realm of activity from the one-on-one physician/patient interaction to the far more complex public arena of social policy, resource allocation, education, and behavior modification. In order to undertake a successful prevention program for a particular disease, one must (1) understand at least some of the causal pathways by which the disorder comes into being, (2) identify subsets of the general population who are at risk of developing this condition, (3) communicate to the target audience in a comprehensible and compelling fashion the appropriate etiologic information and the need to act upon it, (4) develop an intervention strategy that can be applied successfully to the target audience, and (5) demonstrate that this intervention alters the natural history of the disease in a significant fashion. Classically, this requires proving that the intervention can actually *reduce mortality rates* for the disease of interest.

The notion that specific cancers might be prevented had its origin over 200 years ago in Percival Potts' paradigm-creating observations regarding the occurrence of scrotal cancers in chimney sweeps. Numerous additional examples of such causal relationships have followed, including tobacco smoking and lung cancer, aniline dyes and bladder cancer, vinyl chloride and hepatic angiosarcoma, etc. Attempts to use this information to reduce the incidence and mortality of particular cancers have been easiest in the occupational setting, where specific exposures are amenable to modification. It has proven much more difficult to influence etiologic pathways in which

L. Nathanson (ed.), Current Research and Clinical Management of Melanoma.

individual human behavior must be changed in order to effect an exposure reduction.

A second level at which one may attempt to reduce disease mortality is that of earlier diagnosis of established disease. The hope here, of course, is that a malignancy found at an earlier stage in its natural history may be more amenable to cure. Sometimes this notion is indeed correct, as is the case in breast cancers detected by mammographic screening, while in other instances this intuitively appealing hypothesis has failed, as is the case for lung cancer screening by chest x-ray and sputum cytology. One major limitation to screening for most neoplasms is that they arise deep within the body, thus requiring an imaging or diagnostic technique of some kind for detection, and these techniques can be costly and associated with significant problems of diagnostic sensitivity and specificity.

Cutaneous malignant melanoma is a disease that now satisfies many of the criteria listed above and has become the subject of much discussion and study regarding both prevention and screening. We have developed major insights into melanoma etiology over the past 20 years that now permit the identification of high-risk subgroups upon which to focus prevention and screening activities. One major advantage for melanoma relative to other malignancies in this regard is its location on the surface of the skin where the cancer and its precursor lesions can be observed directly. New insights into the biology of melanoma have provided a firm basis for hypothesizing that earlier diagnosis may produce significant decreases in melanoma mortality. The melanoma model developed by Clark and colleagues has permitted the identification of melanoma in situ as a distinctive entity that lacks metastatic potential and has identified two critical biological forms of invasive melanoma: radial growth phase and vertical growth phase [1,2]. The former represents a stage in the development of invasive melanoma in which the malignant cells lack the ability to metastasize. In their studies, the University of Pennsylvania investigators have reported an actual 8-year survival of 100% in their patients with radial growth-phase melanoma [3]. This lesional progression model of melanoma development provides a solid scientific foundation for testing whether screening for melanoma can, in fact, reduce melanoma mortality. This chapter will review our current state of knowledge regarding persons at high risk of melanoma, the embryonic field of chemoprevention as it relates to melanoma, and efforts to undertake melanoma education and screening.

2. Persons at high risk for melanoma

2.1. Hereditary melanoma

The first known English language description of a patient with cutaneous malignant melanoma was, in fact, a familial aggregation of this disease [4].

In this seminal report, Norris formulated two of the hypotheses that have dominated contemporary etiologic research in melanoma, i.e., that this condition has an hereditary component and that the presence of numerous moles on the skin of patients plays an important pathogenetic role. One hundred and thirty-two years passed before Cawley made a similar observation [5], triggering a series of similar, descriptive reports of melanoma-prone families.

In 1983, the first formal genetic analysis of a series of melanoma kindreds was reported. Using maximum likelihood segregation analysis techniques, these data documented an autosomal dominant mode of inheritance for familial melanoma [6]. This analysis was done in families affected by both melanoma and dysplastic nevi (see below). While the initial report could not confirm an autosomal dominant mode of inheritance for dysplastic nevi, subsequent analyses of these data indicated that melanoma and dysplastic nevi represented pleiotropic manifestations of a single autosomal dominant gene [7].

Subsequent reports by other investigators have confirmed the autosomal dominant basis for familial melanoma [8]. The NCI/Penn analysis also suggested by linkage techniques that a gene responsible for the hereditary melanoma/dysplastic nevus trait might be located on the short arm of chromosome 1, in proximity to the Rh blood group locus [6]. Subsequent studies by these investigators employing linkage analysis with additional polymorphic markers (both conventional and restriction fragment length polymorphisms) resulted in assignment of a melanoma/dysplastic nevus gene to the region flanked by the polymorphic markers D1S47 and PND in chromosomal band 1p36 [9]. The multipoint lod score was 5.42 (i.e., the odds were 260,000 to 1 in favor of linkage). Several attempts to confirm this chromosomal assignment by other investigators have thus far been negative [10–12]; both etiologic and diagnostic heterogeneity have been invoked to explain this discrepancy (see ref. 13 for more detailed discussion). Definitive resolution of this controversy awaits a confirmatory trial in which the same case eligibility and diagnostic criteria employed in the original NCI/Penn study are replicated.

Controversies regarding the specific chromosomal assignment of the melanoma/dysplastic nevus gene aside, there is no debate regarding the autosomal dominant genetic mechanism for familial melanoma. As is to be expected, the risk of melanoma is extraordinarily high for members of these unusual kindreds, who are thought to account for approximately 8% of all melanomas (EL Harris, MH Greene, MA Tucker, and D Anderson, unpublished data). In the original NCI study of 14 high-risk families, the prospective relative risk (RR) of melanoma among family members with no prior melanoma was *150*, while the relative risk for melanoma among family members who had had a prior melanoma was *500* [14]. A prospective survey of these family members revealed that melanomas developed only in family members with dysplastic nevi, in whom the cumulative lifetime probability

of developing melanoma approached 100%. Thus, members of melanoma-prone families represent the subgroup of patients at highest risk of melanoma currently identifiable. Within those families, persons with dysplastic nevi who have had one or more prior melanomas represent the very highest risk group. It is worth noting that a positive family history for melanoma has emerged as one of the most important melanoma risk factors in melanoma case-control studies. For example, in the Australian survey of Holman and Armstrong, a multivariate model consisting of nevus number, pigment profile, and family history comprised the major melanoma risk factors. The relative risk (RR) of melanoma for persons reporting one or more family members with melanoma was 2.4 [15].

2.2. Other genetic disorders

2.2.1. Xeroderma pigmentosum. Xeroderma pigmentosum (XP) is a rare autosomal recessive disorder (affecting approximately 1 in 250,000 persons), characterized by sun sensitivity and a dramatic predisposition to skin cancers at an early age. This multisystem disease also manifests neurological deterioration in some patients. Its underlying cellular abnormality is defective excision repair of ultraviolet radiation-induced DNA damage (particularly thymine dimers). Most commonly, these patients develop early-onset, multiple basal and squamous cell cancers of the skin. However, a significant predisposition to malignant melanoma has also been documented. An early literature review of 420 XP patients revealed 19 (4.5%) with melanoma [16]. Of these, nine developed multiple primary melanomas, an observation reminiscent of hereditary melanoma, in which 30% of affected patients develop more than one primary melanoma [14]. A dramatic example of this phenomenon was reported by Tullis et al., who described a young woman with XP who developed 11 independent primary melanomas [17]. Interestingly, this patient also displayed multiple atypical nevi, which were classified as dysplastic nevi, suggesting a possible overlap between these two genetic syndromes.

Kraemer's most recent literature review encompasses 830 published cases, identifies 37 XP patients with melanoma (5%), and suggests that the risk of melanoma is more than 2000 times greater than that expected on the basis of general population rates [18]. Melanomas occur at a median age of 19 years, about 10 years later than do the basal and squamous cell carcinomas in these patients, and have a striking predisposition towards occurring on the face, head, and neck. These sun-exposed sites account for 65% of melanomas in XP patients, compared with 20% in unselected melanoma patients. Although formal, quantitative cohort studies defining the magnitude of melanoma risk in XP patients have not been done, the reality of this association is beyond doubt.

Additional weight in support of the causal nature of this association is the presence of melanoma in both Japanese [19] and Chinese [20] XP patients.

Ordinarily, melanoma is vanishingly rare in Asian populations. In the Japanese experience, melanoma was observed in 18 (6.9%) of 262 XP patients. It has been suggested that certain XP complementation groups may be more melanoma prone than others. Jung et al. suggest that group D patients may be at greater risk [21], with 10 of 11 group D XP patients in their series developing melanoma. Similar claims have been made for group C and XP variants. XP patients certainly would be excellent candidates for chemoprevention trials, though the rarity of this condition is a major limitation.

2.2.2. Neurofibromatosis. Neurofibromatosis (NF) is an autosomal dominant neurocristopathy with a diversity of manifestations, including a predisposition to cancer, particularly cancers of neural crest origin. Malignant Schwannoma is the most frequent cancer reported in this syndrome; brain tumors, sarcomas and pheochromocytomas also occur in excess. This disorder includes a variety of pigmentary abnormalities including cafe-au-lait spots, axillary freckles, and atypical pigmented nevi. Melanocytes are embryologic derivatives of the neural crest. Together, these facts render plausible the hypothesis that melanoma should be among the cancers developing in patients with NF. No formal cohort studies have been performed to test this hypothesis. A 1981 literature review documented 22 patients with NF who had developed melanoma, but the authors concluded that this did not represent an excess [22].

A review of a series of 900 patients with melanoma yielded only one patient who also had NF [23]. Case reports of this association continue to appear, some of which contain hints that this juxtaposition may be more than coincidental. Several report the occurrence of melanoma in structural contiguity with characteristic NF lesions such as a cafe-au-lait spot [24], a neurofibroma [25], and a congenital nevus spilus [26]. In addition, melanoma has been described in both black [25] and Japanese [27] NF patients, racial groups in whom melanoma is ordinarily quite rare. This possible association awaits further elucidation.

2.2.3. Oculocutaneous albinism. Oculocutaneous albinisim is an autosomal recessive disease in which melanin synthesis is either abnormal or absent. At least 10 different varieties of this syndrome have been reported. Although affected patients produce little or no melanin, their melanocytes are physically intact. These patients have a very high incidence of basal and squamous cell cancers of the skin, but the occurrence of melanoma seems to be rather uncommon. Schulze et al. reviewed the literature, compiling 16 instances in which patients with oculocutaneous albinism developed melanoma and added two new cases of their own [28]. They cited the low incidence of melanoma in cohorts of 350 and 1000 albino patients in which 1 and 0 melanomas, respectively, were observed. The reason for this low incidence is unclear. It may reflect under-reporting or difficulties in diagnosing

what are usually amelanotic melanomas (87% of reported cases). Of interest is the occurrence of dysplastic nevi in two patients with albinism [29,30].

2.3. Nevi

2.3.1. Congenital melanocytic nevi. The degree of melanoma risk associated with congenital melanocytic nevi (CMN) comprises an area of great controversy and uncertainty at the present time. These lesions, which by definition are present at birth, have been subclassified by size into three categories: small (<1.5 cm in diameter), medium (1.5–20.0 cm), and large (>20.0 cm). The large lesions may cover a significant portion of the skin surface and are often designated giant congenital nevi, bathing trunk nevi, or garment nevi. CMN have been reported in approximately 1% of newborns, and the vast majority of these are of the small variety [31]. Large CMN are much less common, occurring in approximately 1 in 20,500 live births [32].

These lesions have a characteristic morphology, consisting of a grossly irregular surface, increased pigmentation with varying shades of brown and hypertrichosis, and a characteristic histology [33]. Although some estimates have placed the risk of developing melanoma in a small CMN in the range of 3- to 10-fold greater than expected [34], the NIH Consensus Conference on Melanoma Precursors concluded that the melanoma risk in such patients is unknown and that there exist insufficient data to recommend prophylactic removal of all such lesions [35]. There are virtually no quantitative data on the melanoma risk in medium-sized CMN.

There is a large case-report literature describing the development of melanoma in patients with large CMN. Two quantitative studies warrant citation in this regard. Lorentzen et al. conducted a retrospective cohort analysis of 151 Danish patients with large CMN ascertained 1915–1975 and followed up through the Danish Health Registry [36]. Three patients died from melanoma in this series, and they estimated that 4.6% of patients with CMN might be expected to develop melanoma. This study has many limitations but it has the merit of being relatively quantitative. Gari and colleagues at NYU have established a registry of patients with large CMN and are conducting prospective melanoma surveillance in this cohort. During a mean of 53 months of follow-up in the first 47 patients registered, only one person has developed melanoma [37]. This is a small cohort, subjected to limited follow-up thus far, but this approach should eventually yield a more precise estimate of melanoma risk.

The NIH Consensus Conference estimates the cumulative lifetime risk of melanoma in patients with large CMN to be in the range of 5–20% [35]. The management of these large lesions is exceedingly complex [31], and no strategy to date has been shown to reduce either the melanoma incidence or mortality rates in affected persons. There is a potpourri of other unusual melanocytic lesions in which melanoma has been reported, including neurocutaneous melanosis [38], Nevus of Ota [39], nevus spilus [40], blue

nevus [41], and Becker's nevus [42]; these entities are too rare to be of practical value in prevention programs.

2.3.2. Common acquired melanocytic nevi. The past decade has seen develop a new awareness of the importance of common acquired melanocytic nevi (CAMN) as determinants of melanoma risk. It has long been recognized that remnants of ordinary junctional, dermal, and compound nevi are observed through the microscope in structural contiguity with the melanomas, which seem to be arising from them. Estimates of the frequency of this phenomenon have varied widely and have been highly influenced by the observer's mind set and the definitions employed. Rates ranging from 18% to 72% have been reported [1], with findings in the 30–50% range being most typical [43].

The role of CAMN as a melanoma risk factor has been convincingly demonstrated by a series of case-control studies performed over the past decade (see Greene [13] for a complete list of studies). These 13 studies have varied in their definition of CAMN, the sites at which lesions were counted, and the methods of enumeration (self-count, physician examiner, interviewer examiner, etc). As a consequence, fairly broad differences in relative risk have emerged, but the overall trend is clear: In most surveys, the number of CAMN has emerged as the single most important determinant of melanoma risk. The reported relative risks range from 1.8 to 53.9 (median: 10.1) for the highest mole count categories. I will cite several examples just to convey the flavor of the data.

In the Western Australia Melanoma Study, the presence of 10 or more palpable nevi on the arms yielded a multivariate odds ratio of 10.4 [15]. The Queensland Melanoma Study documented a multivariate odds ratio of 22.8 for the presence of nevi on the arms, and the risk increased as the number of nevi increased [44]. The San Fransisco Melanoma Study documented a gradient in melanoma risk as a function of mole number: 11–25 moles, RR = 1.6; 26–50 moles, RR = 4.4; 51–100 moles, RR = 5.4; 101 + moles, RR = 9.8 [45]. A population-based melanoma study in Denmark revealed a similar pattern [46]. Of particular importance is the fact that the melanoma risk associated with nevus number is independent from that seen with other melanoma risk factors. It has been estimated that number of CAMN may account for 29–79% of all melanomas [47]. These data indicated that one simple way to select a high-risk population as a target for melanoma education and screening efforts would be to focus upon persons with large numbers of ordinary nevi. I will return to this notion subsequently.

2.3.3. Dysplastic melanocytic nevi. Dysplastic melanocytic nevi (DMN) were initially identified as important melanoma precursors in the context of familial melanoma [48,49]. This topic has been reviewed extensively [13,14,50,51]. The etiologic relationship between DMN and hereditary melanoma has been demonstrated most convincingly in the prospective follow-up of the 14

melanoma-prone kindreds described by the NCI/University of Pennsylvania group [14]. In this survey, new melanomas developed exclusively in family members with DMN; *no* prospective melanomas occurred in family members with clinically normal skin. The melanoma risks in this context have been cited above. Careful pathology review of these prospectively diagnosed melanomas revealed that 92% developed in histologic contiguity with remnants of a DMN, documenting that these nevi are true structural precursors of the melanomas detected. These findings have been confirmed recently in a larger cohort of families with longer follow-up (MA Tucker, personal communication).

It subsequently became apparent that DMN occur in melanoma patients with no family history of melanoma [52] and in persons with neither a personal nor a family history of melanoma [53]. Detailed histologic studies have demonstrated a DMN at the margin of approximately one third of *unselected* melanoma specimens [43]. Epidemiologic studies that include direct examination of the skin have now been performed, yielding DMN prevalence estimates that range from 6% to 55% (median 34%) in case subjects and from 0% to 17% (median 7%) in control subjects [45,47,54–58]. Patients with DMN also have greater numbers of common acquired nevi than do persons without DMN [45,56,58,59]. Analyses directed towards separating the contribution of each class of nevi to melanoma risk suggest that these may be independent risk factors, although the findings are not uniform. It is estimated that from 29% to 49% of nonfamilial melanomas may be attributable to DMN as a risk factor [47].

Kraemer et al. estimated the risk of melanoma in patients with nonfamilial DMN to be seven times that expected in the general population [60]. Rigel et al. prospectively monitored 281 patients with nonfamilial DMN (and no prior personal history of melanoma). During 27 months of follow-up, they detected three in-situ and one invasive melanoma. The risk of invasive melanoma was 16 times that expected [61]. Although this study is limited by its small sample size and brief follow-up, it clearly demonstrates that patients with nonfamilial DMN are at increased risk of melanoma. The risk observed is several orders of magnitude less than that reported for patients with familial DMN, but it is very substantial nonetheless. Quite clearly, patients with either type of dysplastic nevi would represent excellent subjects for melanoma prevention and screening activities.

2.3.4. Lentigo maligna. Lentigo maligna is an indolent proliferation of atypical intraepidermal melanocytes that appears on the chronically sun-exposed skin of elderly Caucasians. This entity was originally identified by Hutchinson in 1892, resulting in the widely used eponym, *Hutchinson's melanotic freckle* [62]. Clinically, it presents as a macular lesion, typically on the face, containing a multiplicity of colors, ranging from tan to brown to dark brown to black; areas of regression or clinically normal skin may be seen within the confines of the pigmented patch, which itself is virtually

always located on sun-damaged skin. These lesions are generally brought to clinical attention when they develop a nodule within their macular substrate, an event that usually heralds the development of invasive lentigo maligna melanoma.

There is some controversy at present regarding whether lentigo maligna is properly considered a precursor to lentigo maligna melanoma [63] or whether it is more accurately thought of as lentigo maligna melanoma in situ [64]. In epidemiologic studies, lentigo maligna melanoma has been most closely linked with chronic, cumulative sunlight exposure in a manner that parallels the sunlight associations observed in nonmelanoma cancers of the skin [65]. There are precious few data regarding the probability of lentigo maligna evolving into invasive lentigo maligna melanoma. Guesstimates on the frequency of this event range from 5% [66] to as high as 33–50% [67,68]. Weinstock and Sober have presented the only quantitative analysis of this issue to date in a study that approaches the question indirectly and makes a number of assumptions [69]. In a series of calculations employing data from the national Health and Nutrition Evaluation Survey I, national SEER tumor registries, and three large melanoma cohorts, they estimate the lifetime probability of malignant transformation starting at age 45 to be 4.7%. Their data suggest, therefore, that the risk of lentigo maligna evolution into full-fledged invasive melanoma is significantly lower than the estimates generally cited in the nonquantitative literature. There is no doubt regarding the fact that such transformation does take place.

The HANES I survey suggests that lentigo maligna is relatively common above the age of 65: 11.7 cases per 1000 persons in the general Caucasian population. While the prevalence of lentigo maligna is high enough to generate reasonable numbers of persons for melanoma intervention programs, the annual rate of malignant transformation may be so low (0.14% per year in persons age 65–74) as to render such patients impractical for chemoprevention efforts.

2.4. Cutaneous phenotype/sunlight

The traditional epidemiology of melanoma is reviewed in detail elsewhere in this book. For purposes of background, let me simply state that light eye color (usually blue), light hair color (blonde or red), fair complexion (Types I, II), a tendency to freckle, inability to tan, and predisposition to sunburn have been consistently identified as important risk factors for cutaneous melanoma [15,44–46,55,58,70]. The prototypic high-risk patient demonstrates what I think of as the 'Irish lass phenotype.' The risk of melanoma is typically 2–3× higher than expected in such persons. These and other observations have long suggested that sunlight plays a central role in the pathogenesis of melanoma. The epidemiologic data regarding sun exposure as a risk factor are quite complex.

Armstrong's authoratative review documents possible roles for both inter-

mittent, intense sun exposure, and chronic cumulative sun exposure, with the former thought to be of greater importance [71]. Severe sunburn at a young age has emerged as a particularly interesting component of this relationship. It is widely accepted that population-wide changes in recreational sun exposure habits have played a major role in the rapidly escalating melanoma incidence rates reported recently [72].

A particularly distressing manifestation of this 'tan is beautiful' attitude is the emergence of tanning parlors and home tanning devices as yet another source of exposure to ultraviolet (UV) light. Exposure to these artificial sources of UV light has been reported to produce chronic actinic injury, freckling, and lentigines. A smattering of case reports has documented the occurrence of melanoma in persons so exposed. There are two excellent epidemiologic studies in which this issue was explored in depth. Swerdlow et al. reported a 2.9-fold excess of melanoma among users of UV lamps and sunbeds, with risk increasing significantly as the duration of exposure increased [73]. Walter et al. confirmed both observations and also noted that domestic tanning devices seemed to have a higher risk than commercial tanning salon exposure [74]. This latter finding may be a result of domestic units more often being emitters of UVB, while salons have shifted towards the use of UVA devices.

One particularly worrisome aspect of this phenomenon is the recognition that exposure to UV light in tanning salons induces measurable immune dysfunction [75]. (See below for more data regarding the increased risk of melanoma in immunocompromised patients.) It is noteworthy that the typical pattern of UV exposure in users of artificial tanning devices mimics exposure characteristics identified as important in epidemiologic studies of melanoma and sunlight, i.e., high dose rate exposures for relatively brief, intermittent periods of time to skin that has been previously unexposed. Cutaneous phenotype, therefore, is a marker that permits identifying an important high-risk group. Interventions that modify UV exposure might be expected to reduce melanoma incidence.

2.5. Prior melanoma

The fact that patients with one melanoma are significantly more likely to develop a second melanoma is illustrated most dramatically in hereditary melanoma. In the original NCI/Penn cohort of 14 families, 30% of melanoma patients developed a subsequent primary melanoma [14]. Persons with one melanoma were 3.3 times more likely to have a new melanoma than were family members with no prior melanoma (RR = 500 vs. RR = 150, respectively). Many authors have reported their experience with second melanomas in large institutionally based melanoma cohorts. For example, Scheibner et al. detected 90 second melanomas in the Sydney cohort of 3128 melanoma patients, a frequency of 2.9% [76]. Most case series of this type suffer from the lack of detailed patient follow-up and the absence of formal quantification

of melanoma risk. Veronesi et al. calculated that a patient with one melanoma is 900 times more likely to develop a second primary than expected from general population rates [77]. This estimate exceeds that reported in hereditary melanoma and must be too high.

A more accurate estimate has been derived by analyzing the development of subsequent cancers in 4693 persons with cutaneous melanoma diagnosed in Connecticut between 1935 and 1982 [78]. There were 30 second melanomas, accounting for 10% of all second cancers seen in this cohort. The relative risk (RR) for a second melanoma was 8.5 overall and was significantly higher (RR = 23) in patients less than age 40 at first melanoma diagnosis. An intensive follow-up study of 384 consecutive patients with melanoma diagnosed at the MD Anderson Hospital 1969–1970 documented a second melanoma relative risk of 22–45, depending on the reference population used to generate the expected number of melanoma cases (E Harris, MH Greene, MA Tucker, and D Anderson, personal communication). As the Connecticut Tumor Registry may not learn of second cancers in persons who leave the state after their first melanoma diagnosis, the MD Anderson risk estimate may be more accurate. Nonfamilial melanoma patients with multiple primary melanomas are significantly more likely to have dysplastic nevi than persons with but one melanoma [79]. Thus, patients with a first melanoma comprise a readily identifiable high risk group that would be suitable for prevention and intervention activities.

2.6. Other cancers

While the diagnosis of one cancer does not, in general, herald an across-the-board increase in the risk of a subsequent second primary of a different type, a series of site-specific associations has become apparent over the years. The basis of these associations is usually either (1) the two cancer types share a common risk factor or etiologic pathway, or (2) treatment for the first cancer induces the second. A series of non-treatment-related associations has been reported for melanoma, and these will be summarized briefly.

A population-based survey of 1973 patients with basal call cancer known to the Swedish Cancer Registry documented that the relative risk of melanoma was significantly elevated for both men (RR = 6.8) and women (RR = 4.2) [80]. This fits with prior epidemiologic studies of melanoma in which a prior history of nonmelanoma skin cancer emerged as a significant melanoma risk factor [45,81,82]. Presumably sunlight exposure plays a role in both neoplasms. Patients with primary brain tumors are 2.1–6.1 times more likely to develop melanoma than general population expectation [78,83]. Both neoplasms are of neural crest derivation; this may provide the etiologic link.

Retinoblastoma (RBL) is another neural tumor for which an excess of subsequent melanoma has been suggested. A literature review suggested

that melanoma accounts for approximately 7% of second malignancies in survivors of RBL [84]. A number of these patients have been reported to have dysplastic nevi. Several different lymphoproliferative neoplasms have been associated with an increased risk of subsequent melanomas. These include Hodgkin's disease (melanoma RR = 6.7–8.0) [85,86], non-Hodgkin's lymphoma (RR = 2.4) [87], and chronic lymphocytic leukemia (RR = 6.7) [88]. The underlying immune dysfunction that accompanies these lymphoproliferative cancers is thought to account for the risk of melanoma. At least some of these patients have dysplastic nevi [86]. A hormonal link has been suggested as the explanation for the excess melanoma risk (RR = 1.4–1.5) observed in women with breast cancer [89,90]. For purposes of focusing education and prevention activities, patients with nonmelanoma skin cancer are probably the most suitable, as they represent a large cohort of persons with an excellent prognosis who are not preoccupied with treatment of their initial cancer.

2.7. Immunosuppression

2.7.1. Organ transplantation. Patients undergoing renal transplantation are at increased risk of subsequently developing several specific types of cancer, including skin cancer in general and melanoma in particular [91–93]. The relative risks for melanoma in this setting are 3.9–5.0. The characteristics of melanoma following renal transplant have been reported in detail [94]. Most patients were treated with azathioprine and prednisone, but melanoma following cyclosporine has been reported as well [95]. Occasional examples of melanoma following bone marrow transplantation have been described [96]. Contrary to clinical intuition, formal decision analysis techniques have suggested that the benefits associated with continuing immunosuppression outweigh the risks for patients who develop primary melanoma in this setting [97].

A surprisingly high proportion of post-transplant melanomas arise in dysplastic nevi [94], suggesting that immunosuppression in a host with melanoma precursors brings with it a particular susceptibility to neoplastic transformation. Thus, it is notable that the sudden appearance of increased numbers of both ordinary [98] and dysplastic [99] nevi in transplant recipients has been reported. A recent case-control study of children undergoing renal transplant documented a significant increase in the number of nevi counted in cases compared with controls, an increase that was strongly correlated with the duration of immunosuppression (CH Smith, personal communication). This suggests that immunosuppression may contribute to the development of nevi as well as melanomas.

2.7.2. Chemotherapy. As noted above, increased risks of melanoma have been reported in patients undergoing or following treatment of Hodgkin's disease [85,86], non-Hodgkin's lymphoma [87], chronic lympocytic leukemia

[88], and cancers of the brain [78,83] and breast [89,90]. Various etiologic mechanisms have been proposed, but a role for chemotherapy-induced immunosuppression has not been excluded. Chemotherapy for childhood cancer has been associated with increased numbers of nevi in cases vs. controls in two separate reports [100, 101], findings analogous to those cited above for the post-transplant patient.

2.7.3. Other immunosuppressed states. A threefold excess risk of melanoma has been observed in persons with various genetically determined immunodeficiency diseases [102] and in patients on chronic hemodialysis [97].

2.7.4. Human immunodeficiency virus disease (AIDS). Since 1985, there have been a flurry of case reports describing the occurrence of melanoma in 12 patients with HIV-1 infection [103–111] and one patient with HIV-2 [112]. No formal cohort analysis has been performed to provide a quantitative estimate of melanoma risk, although it has been estimated that the melanoma incidence rate is 100 cases per 100,000 patients with AIDS [107], which, if correct, is certainly quite elevated. As noted in transplant recipients and chemotherapy patients, the sudden appearance of multiple dysplastic nevi has been reported in HIV patients [113]. One patient developed 10 primary melanomas and multiple dysplastic nevi [111]. While an excess risk of melanoma in HIV patients has not yet been proven, the documented melanoma predisposition of immunocompromised patients makes this hypothesis quite plausible.

2.7.5. Psoriasis/PUVA. The use of 8-methoxypsoralen plus irradiation with UVA (PUVA) in patients with psoriasis has been shown to increase the risk of atypical pigmented lesions (the 'PUVA lentigo') and squamous cell carcinoma of the skin [114]. Laboratory studies have suggested that PUVA may accelerate the growth of melanoma cells transplanted into mice [115]. In spite of these theoretical concerns, there have appeared to date only a handful of case reports of melanoma in psoriasis patients treated with PUVA (and usually multiple other agents as well). A formal cohort analysis has failed to reveal a melanoma excess in this context (three melanomas observed vs. 2.05 expected) [116].

2.8. Hormones

The development of hyperpigmentation during pregnancy and in conjunction with the use of oral contraceptives, coupled with the notion that pregnancy may adversely impact the natural history of established melanoma, have provided basis for concern that reproductive factors, oral contraceptives, and estrogen replacement therapy might comprise significant risk factors for melanoma. While occasional weak positive findings have emerged from a few of the many studies performed to test these hypotheses, overall no

reproducible, consistent association has been observed between any of these hormonal factors and melanoma risk [117]. A role for oral contraceptives in melanoma pathogenesis has been ruled out most convingingly [118,119].

Hormone replacement therapy has been less thoroughly eliminated from concern. The most recent and largest study to date detected a 50% excess of melanoma (RR = 1.50) that just barely reached statistical significance [120]. This finding gains some support from the observation that oophorectomy may *protect* against the risk of melanoma [121]. However, the authors caution that chance and possible confounding by solar exposure could not be ruled out as possible explanations for this modest melanoma excess [120].

While most studies of various reproductive characteristics of women have shown no significant association with melanoma risk [117,118], there is a lingering concern that pregnancy itself may trigger the development of melanoma in some women. One recent interesting observation in this regard is Ellis' report that pregnancy and hormone replacement therapy (but not oral contraceptives) are associated with increased rates of nevus change in patients with dysplastic nevi [122]. This requires additional study but, for now, we cannot identify a subgroup of women who clearly experience increased risk of melanoma on the basis of a hormonal risk factor.

2.9. Occupation

As noted in the introduction, cancer control efforts have great potential effectiveness in the occupational setting if specific exposures can be identified as disease risk factors. There exists a fairly extensive but rather spotty literature on occupational risk factors for melanoma. A threefold excess of melanoma has been documented among workers at the Lawrence Livermore Laboratory, but no specific risk factor has been isolated in spite of extensive study [123]. Printing workers [112,125], chemists [126–128], telecommunications workers [127,129], and oil refinery workers [130] have been tentatively identified as at increased risk of melanoma, but in no instance has a specific exposure been linked to the development of melanoma, with the possible exception of cutting oils [131]. This subject has been reviewed in detail [132]. Melanoma risk reduction in the workplace will have to await definitive identification of specific occupational exposures.

2.10. Summary

This represents an overview of the data to date regarding the identification of persons at high risk of cutaneous melanoma. From a practical point of view, population subgroups that are most suitable for melanoma prevention and screening efforts are hereditary melanoma patients and their family members, persons with dysplastic nevi or large numbers of common acquired nevi, those who have had a prior melanoma or nonmelanoma skin cancer, and individuals with a susceptible cutaneous phenotype. Let us now consider

what chemoprevention has to offer as a strategy to reduce melanoma incidence and mortality.

3. Chemoprevention

Chemoprevention as a melanoma risk-reduction strategy would require the administration of an oral or topical medication that somehow modifies disease susceptibility. Such a medication would need to be relatively nontoxic, given the likely requirement for long-term administration. The cost of the medication would influence significantly the practicality of such an approach, and the drug schedule/route of administration would have a major impact on patient compliance. Thus, a satisfactory chemopreventive agent would require proven safety in long-term administration, low cost, and an infrequent, convenient schedule of administration.

3.1. Sunscreens

Topical sunscreens represent a chemopreventive modality that is already widely available. Twenty-two compounds have been classified as 'safe and effective' sunscreens by the U.S. Food and Drug Administration, including para-aminobenzoic acid (PABA) and its esters, benzophenones, cinnamates, anthranilates, and salicylates [133]. These agents absorb ambient UV light. Physical sunscreens (containing particulate materials that reflect and scatter UV light) include medications containing zinc oxide, titanium dioxide, magnesium silicate, ferric chloride, kaolin, and ichthyol [134]. Given the likely etiologic role of UV light exposure and sunburn in the development of melanoma, common sense would dictate that reducing the amount of UV light that reaches the epidermis should lower the risk of melanoma. While sunscreens clearly reduce sun-induced erythema and chronic actinic injury [135], there is as yet no objective, quantitative evidence to document that their use is associated with a decrease in either melanoma incidence or mortality.

With the exception of cutaneous rash, topical sunscreen products are well tolerated; there are no data to suggest that they produce significant long-term toxicity. They are cumbersome to use on a regular basis, requiring at least daily application when sun exposure is anticipated, and reapplication following swimming or exercise-induced perspiration. The physical sunscreens are cosmetically less acceptable. An aggressive program of sun avoidance and sunscreen application has been shown to reduce the rate at which actinic damage and nonmelanoma skin cancers develop in patients with xeroderma pigmentosum [18,136]. Given the balance of costs and benefits, a similar program would certainly be reasonable for persons at high risk of melanoma and might well be associated with significant reductions in melanoma risk.

Total sunlight avoidance in the melanoma setting is undoubtedly too

stringent a prescription. Minimizing exposure to the mid-day sun and scrupulously avoiding sunburn, coupled with liberal applications of sunscreens with a high solar protection factor (SPF >14) rating, would seem to comprise a prudent program given our current state of knowledge. Given recent data regarding the possible role of UVA in the pathogenesis of skin cancer [137], broad-spectrum sunscreens that filter both UVA and UVB are currently the product of choice. Some insight into the potential benefit from widespread sunscreen use can be gained from the analysis of Stern et al., which suggests that the regular use of sunscreens with SPF >14 by all children during the first 18 years of life could reduce the risk of nonmelanoma skin cancer by 78% [138]! Future epidemiologic studies of both melanoma and melanocytic nevi should evaluate sunscreen use in cases and controls in an effort to formally document whether their use confers measurable protection against lesion development.

3.2. Retinoids

Vitamin A and its analogs are well known for their activity in both the treatment and prevention of cancer [139,140]. Modest therapeutic activity against metastatic melanoma has been observed [141], and some evidence suggests a protective effect against skin cancer [142]. Retinoids inhibit the in vitro growth of cultured melanoma cells [143] and promote the differentiation of melanoma cells into mature melanocytes [144]. It was with this information in mind that investigators at the University of Arizona treated three dysplastic nevus patients with topical tretinoin (all-trans retinoic acid, vitamin A acid, Retin-A). Two of three patients responded with clinical fading of the treated lesions and histologic resolution of melanocytic dysplasia [145]. In an effort to simplify retinoid therapy and to broaden it to be systemic in scope, these same investigators then treated 11 dysplastic nevus patients with oral isotretinoin (13-cis retinoic acid), 40 mg twice daily for 4 months. No response to therapy was seen [146]. Their most recent study was a double-blinded, randomized, placebo-controlled trial of topical tretinoin [147]. Twenty-one patients were randomized; five treated patients and 11 controls were evaluable. Three of the five treated patients showed striking clinical and histologic responses to therapy; in 7 of 15 treated nevi the melanocytic dysplasia had either disappeared or resolved by the end of the study.

Investigators at the University of Pennsylavania have confirmed the activity of topical tretinoin in a study of 20 patients with hereditary melanoma/dysplastic nevi (A. Halpern, personal communication). While tretinoin showed clear biologic activity in these studies, Edwards et al. were pessimistic about the widespread applicability of this regimen, as it is cumbersome to apply to large numbers of nevi and is associated with significant local toxicity in some patients. Further, several patients developed nevus recurrences at treated sites when seen in follow-up 1 year after the study ended. Possible mechanisms of action for tretinoin were reviewed [146] and include changes in gene transcription and protein synthesis, cell

membrane changes, modification of both cellular and humoral immunity, and direct cytotoxicity. Retinoids may exert some of their important biological effects through inhibition of ornithine decarboxylase (see below) [148]. The discrepancy between the oral and topical studies may be accounted for by the fact that 13-cis retinoic acid was used in the former, while all-trans retinoic acid was used in the latter. With the recent availability of an oral form of all-trans retinoic acid, it may well be worth attempting another oral trial using what appears to be the more active stereoisomeric form.

Beta-carotene and alpha-tocopherol are intracellular antioxidants that quench free radicals. They each are capable of inhibiting the in vivo development of UV-induced skin tumors in mice [149,150]. Epidemiologic studies relating these who compounds to melanoma risk are inconsistent, with some suggesting a protective effect for high serum levels or consumption [151,152], while others do not [153]. The toxicity of these two agents is minimal, making them acceptable candidates for large-scale clinical trials in humans. The Southwest Oncology Group is about to activate chemoprevention protocol 9025, a randomized, phase II feasibility/pilot study of beta-carotene plus alpha-tocopherol vs. placebo in subjects at high risk of melanoma. Patients with multiple ordinary nevi, clinically atypical ('dysplastic') nevi, or a prior thin melanoma will be eligible to participate. Hopefully, this will be but the first of a series of melanoma chemoprevention trials, with new studies coming on line as new candidate chemoprevention agents are identified. This is clearly an exciting avenue for future research.

3.3. Diflouromethylornithine

Alpha-diflouromethylornithine (DFMO) is an enzyme-activated irreversible inhibitor of ornithine decarboxylase, the rate-limiting enzyme in polyamine biosynthesis. It is active in the inhibition of tumor development in a number of experimental models, including the induction of murine skin cancer by DMBA and TPA, colon cancer induction by 1,2 dimethyl hydrazine, rat mammary cancers induced by 1 methyl 1 nitrosourea, and rat urinary bladder cancers induced by N-butyl-N-(4-hydroxybutyl) nitrosamine [154]. In a fascinating animal experiment, DFMO markedly inhibited both the cutaneous carcinogenesis and immunosuppression induced by UVB in mice. While this compound is in human clinical trials as a possible preventer of urinary bladder cancer, it has not yet been studied as a chemopreventive agent in skin cancer. A trial of DFMO in persons at high risk of melanoma merits serious consideration.

3.4. 5-Flourouracil

Bondi et al. applied topical 5% 5-FU to six dysplastic nevi in a single patient twice daily for 5 weeks [155]. All six lesions became inflamed, ulcerated, and then disappeared. Biopsies at the treated sites revealed no evidence of residual melanocytic dysplasia; four control nevi did not change. 5-FU is

widely used in the treatment of the preneoplastic lesions of nonmelanoma skin cancer, but this interesting evidence of biological activity against a precursor of melanoma has never been pursued systematically. Ryan et al. reported their experience with the topical application of 5-FU to melanomas in five patients and to dysplastic nevi in two others [156]. They confirmed Bondi's report of activity in dysplastic nevi and documented a surprising degree of effectiveness in established melanoma as well. Additional clinical trials might prove worthwhile for this agent.

3.5. Vaccines

Immunotherapy has a long and checkered history in the management of malignant melanoma. Such research has been spurred by various tantalizing observations, such as the occasional spontaneous remission in a patient with advanced melanoma, various seemingly host vs. melanocyte responses (such as the occult primary, areas of regression within primary melanomas, the presence of a mononuclear cell infiltrate in both primary melanomas and dysplastic nevi, and vitiligo), the presence of antimelanoma antibodies in the serum of melanoma patients, and the identification of 'melanoma-specific' antigens on the surface of melanoma tumor cells.

Until recently, most clinical attempts to manipulate the immune response to prevent melanoma recurrence or to treat advanced disease have been unsuccessful. Recently, however, results of a number of early trials of melanoma vaccines in humans have been more encouraging. Use of these vaccines has yielded some evidence of regression in established melanoma metastases and has produced some hints of prolonged survival in patients at high risk of recurrence [157–162]. Of particular interest are animal data that suggest mice immunized with melanoma vaccines survive the injection of doses of live melanoma tumor cells that routinely kill mice that are not immunized [163–165].

These vaccines' ability to prevent melanoma in mice raise the possibility that vaccines may ultimately play a role in melanoma prevention in humans as well. This notion gains plausibility in the light of data that show dysplastic melanocytes share various cell surface markers with malignant melanocytes [166–168], suggesting that an effective antimelanoma vaccine might alter the risk of melanoma in persons with dysplastic nevi. Such an approach to melanoma risk reduction is probably in the distant future, awaiting the development of a melanoma vaccine that is proven safe and effective. In the meantime, this potential use in the prevention of melanoma provides an additional rationale for supporting ongoing efforts at vaccine development.

4. Screening for melanoma

In January 1992, the NIH convened a consensus development conference, entitled The Diagnosis and Treatment of Early Melanoma [169]. Question 5

to which the conference addressed itself was, 'What is the role of education and screening in preventing melanoma morbidity and mortality?' It produced the following rather dramatic statement: 'There is sufficient evidence to warrant screening programs for melanoma in the United States!' This position contrasts with that of the Australian Cancer Society, which asserts 'it cannot recommend to the general public that they be regularly screened for melanoma by a doctor,' [170] in spite of Australia's much higher melanoma incidence rates. How can these positions be reconciled? What do we know about melanoma screening in 1992?

4.1. Conceptual and biological rationale

Two types of disease prevention activities can be undertaken: (1) primary prevention, which aims at reducing disease mortality by reducing disease incidence through risk factor modification, and (2) secondary prevention, in which mortality is lowered by early disease detection, thereby imposing chances of long-term, disease-free survival. How can one document the effectiveness of prevention programs? The gold standard is a prospective, randomized clinical trial in which active intervention is tested against observation and that demonstrates *reduced mortality* in the 'treated group.' This study design is required because it alone avoids the many methodological and statistical biases inherent in other study designs. A full discussion of these issues is beyond the scope of the current presentation, but the major biases include lead-time bias, length bias, self-selection bias, and diagnostic bias [171]. In general, these biases tend to inflate artifactually the estimated benefit of screening. The likelihood is small that a randomized melanoma prevention trial will ever be done (with the possible exception of chemoprevention studies). Such projects are large, complex, expensive, and require either very large cohorts or many years of surveillance to produce statistically reliable results.

Other methods for addressing this issue do exist, but they are more subject to the various types of statistical bias mentioned above and, thus, yield results that must be interpreted with caution. As summarized by Koh et al. [172], these options include: (1) testing whether morbidity and mortality rates drop after the introduction of an intervention program, (2) comparing rates of advanced disease or death in people who are screened with those who are not, (3) retrospective case-control studies testing whether risk of death is increased by absence of screening (such a study is now underway in Connecticut with the intent of enrolling 1250 melanoma cases and 1250 general population controls [187]), and (4) conducting ecological correlations between the incidence of disease and the use of screening among different populations. Another option is to substitute an intermediate end point, such as melanoma tumor thickness, for the optimum end point of mortality. This approach is based on the assumption that, since thinner melanomas have a better prognosis, a shift in melanoma microstage towards a higher proportion

of 'thin' lesions implies that a reduction in mortality will follow. This technique has been used widely and will be discussed in greater detail below.

Under what circumstances can a screening program be expected to achieve success? In general, the following criteria must be met. The disease of interest must have incidence and mortality rates that are high enough to represent an important public health concern. The natural history of the disease must be understood, and there should exist an early or latent symptom-free period during which screening can be applied. There must exist a reliable screening test, preferably one that is safe, cheap, and acceptable to the target population. Effective treatment for the disease must be available, with evidence in hand that early diagnosis results in meaningful decreases in disease-associated morbidity and mortality. Finally, the health care system must be prepared to respond to the demand for diagnostic and treatment services that result from communitywide intervention programs. How does melanoma measure up to these criteria as a candidate disease for systematic education and screening activities?

The public health dimensions of cutaneous melanoma are well known and are reviewed elsewhere in this volume (see Chapter 2). Suffice it to say that the incidence of melanoma is rising faster than any other cancer in the United States, and the rate of increase in mortality rates for melanoma is second only to lung cancer [173]. The relatively young average age at melanoma diagnosis results in a loss of productive life that is disproportionately high relative to its incidence [174]. By this measure, melanoma represents an appropriate consideration for active intervention.

As noted in the introduction, the last decade has seen major new insights into the biology of cutaneous melanoma. The identification of dysplastic nevi as important melanoma precursors has resulted in a clearer understanding of the stepwise nature of melanoma tumor progression, as elegantly described by Clark and coworkers [1–3]. This has resulted in a more precise definition of melanoma in situ, an entity that had long plagued dermatopathologists. It also produced the observation that radial growth phase melanoma represents a definable stage in melanoma tumor progression, characterized by invasive disease that lacks the ability to metastasize. The evolution through the various stages of melanoma tumor progression is generally measured in years. Thus, melanoma in situ and radial growth phase melanoma represent the vital latent phase of disease during which there is an opportunity to intervene before metastasis has occurred.

The screening test in this case is visual inspection of the skin, a technique readily accepted by patients and one dramatic in its simplicity in this high-tech era of molecular biology and magnetic resonance imaging. How reliable is this test? There are three statistical parameters that are employed to answer this question: (1) *Sensitivity*: This describes how well a test succeeds in identifying people with the disease of interest (the number of true positive tests divided by the number of persons tested who actually have the disease). (2) *Specificity*: This describes how well the test identifies people who *don't*

have the disease (the number of true negative tests divided by the number of persons tested who really do not have the disease). (3) *Positive predictive value*: This is the number of persons who actually have the disease, divided by the number of people who test positive [175].

In applying a screening test to a population, one attempts to balance the occurrence of false-positive and false-negative test results. To avoid overlooking patients who actually have the disease, one prefers high sensitivity and low specificity; this brings with it the risk of false-positive results. To avoid the anguish of false-positive findings, lower sensitivity and higher specificity may be employed, but the downside here is false-negative results, in which some persons with disease are overlooked. The positive predictive value of a screening test is strongly influenced by the prevalence of disease in the screened population. One technique, therefore, for improving the positive predictive value of the test is to apply it to an 'enriched population,' i.e., to persons selected *because* they are at high risk rather than simply screening the general population. As noted above, this can clearly be accomplished for malignant melanoma.

There exist in the literature only a few estimates of these parameters for the clinical diagnosis of malignant melanoma [172,176–180]. In these series, sensitivity estimates range from 73% to 97%, specificity estimates range from 92% to 99%, and estimates of positive predictive value range from 35% to 80%. The study of Kopf et al. represents the best contemporaneous experience by university-based dermatologists with a special interest in melanoma. The values for the three parameters were 77%, 99%, and 80%, respectively [179].

The Massachusetts experience with the American Academy of Dermatology screening program is a more reasonable test of the real world. It yielded a sensitivity of 97% and a positive predictive value of 35–40% [180]. The study design did not permit evaluating specificity. This is clearly an area where more information is needed but, in general, these parameter estimates are similar to those reported for other screening procedures [175]. Experience to be cited below suggests that clinicians can be trained to do this task, although it is clear that dermatologists do better than other physicians in appropriately classifying pigmented cutaneous lesions [181].

Effective and relatively simple treatment (i.e., surgical excision) is available for early melanoma, but we lack definitive proof that early diagnosis results in reduced mortality. The appropriate randomized trial has not been done and probably never will be. Supporters of melanoma screening efforts point to the reproducible shifts in melanoma microstage towards thinner lesions, which almost invariably accompany the introduction of screening (see below for details), as evidence of benefit. While very encouraging and logically compelling, such observations are fraught with potential bias. Nonetheless, such data have persuaded many people to endorse screening efforts in general and large screening trials in particular.

Can our health care system cope with the demand for increased diagnostic

and treatment services that would accompany the wide-scale introduction of melanoma screening in the general population? Probably not at the present time, but experience (summarized below) documents that with appropriate planning this can be accomplished and, in fact, has been accomplished in such widely diverse locales as Queensland, Australia, Glasgow, Scotland, Italy, New Mexico, and Austria [182–186]. Thus, except for not yet having proven that screening reduces mortality from melanoma (a significant shortcoming), the criteria for effective screening are well met for this disease. Let us now consider the results of screening programs to date, and review who should be screened and how educational programs can be applied to melanoma.

4.2. Screening programs

The process of evaluating the skin to identify early melanomas can be accomplished in many ways, including self-examination, examination by relatives or friends, skin evaluation during the course of ongoing medical care for other health problems; examination in the workplace; health fair screening; periodic formal screening programs at the local, state, or national level; or carefully orchestrated, large-scale sustained programs, usually regional or nationwide in focus. Several detailed reviews have summarized these efforts [172,187,188], which in the United States began with the pioneering activities of P.E. Weary in Virginia [189] and is best represented today by the annual nationwide American Academy of Dermatology (AAD)-supported melanoma/skin cancer screening programs [180]. In the aggregate, these ad hoc efforts have demonstrated that the public can be reached and stimulated to participate and that previously unrecognized melanomas can be detected successfully. The AAD program is limited by virtue of being a once-yearly effort, conducted only in those locales where volunteer dermatologists have organized to support the project. Many areas in the country do not participate, there is no formal mechanism of follow-up to ensure that persons referred for further evaluation actually follow through as recommended, and educational activities in between screening days are not systematic. While tens of thousands have been screened by the AAD, this program thus far has been a service rather than a scientific exercise.

At some sites, specific scientific studies have been incorporate into the AAD program, and these have produced valuable data. Koh's active follow-up of the Massachusetts AAD cohort yielded the information on test sensitivity and positive predictive value cited earlier [180]. Rigel et al. used the Manhattan AAD cohort to document the importance of total body skin examination as a screening tool: 13 of 14 melanomas detected were on anatomic sites normally covered by clothing [190]. Patients having complete skin examinations were 6.4 times more likely to have a melanoma detected than those having partial exams. AAD-based surveys in both Massachusetts and Rhode Island demonstrated that screening program participants were, in fact,

at increased risk of skin cancer by risk factor analysis and, thus, had appropriately selected themselves for screening [191,192].

There are few, if any, quantitative data regarding the utility or efficacy of self-examination as a screening technique. The case-control study of Berwick et al. in Connecticut will provide the first real information in this regard [193]. Screening for melanoma in the workplace is a strategy that has been underutilized but that has great potential. An excellent example of this approach can be found in the experience of the Lawrence Livermore National Laboratory.

In the early 1980s, a fourfold melanoma excess was identified among the 10,000 workers at this high-tech research laboratory [194]. In spite of intensive epidemiologic study, the basis for this excess has defied elucidation. Concurrent with attempts to unravel the etiology of this melanoma cluster, the Health Services Department of the Laboratory mounted an aggressive intervention program aimed at early melanoma detection and treatment. The program began in 1984 and adopted a multitiered strategy of educating employees, management, and local health care providers; self-examination and mole counting; and an on-site melanoma clinic for dermatologic examination and treatment [195]. Since 1984, all melanomas diagnosed have been thin tumors, with an anticipated survival of 95–100%. The median tumor thickness is *zero*, i.e., the majority of these lesions were melanoma in situ. Comparison of the melanomas diagnosed among screened workers with melanomas presenting spontaneously in the adjacent, nonemployee community has shown that median melanoma thickness has declined significantly more rapidly among Livermore employees. While no decrease in melanoma mortality has been described (yet) in this cohort, these data suggest a beneficial impact of the screening program. As wellness or health maintenance programs become an increasingly frequent feature of corporate health insurance programs, the opportunity to conduct workplace screening for melanoma should steadily escalate.

The granddaddy of populationwide melanoma screening is the Queensland Melanoma Project, which began in 1963 [182,196]. The project began with a population-based retrospective survey of melanoma incidence and mortality over the years 1945–1963. This study documented formally Queensland's high melanoma incidence rate (at the time, 16.5 per 100,000 per year) and laid the foundation for an ongoing statewide melanoma registry that provided current morbidity and mortality data. An educational campaign was launched, focusing upon both professional and public education. The professional component included paramedical personnel and increased instruction on melanoma in the medical school curriculum. The public was reached through brochures, posters, public meetings, and lectures, and all forms of the media. The relationship between sunlight exposure and melanoma risk was stressed, particularly in the schools, where children became a major focus of the campaign. The educational effort was continuous and ongoing, as decreases in these activities seemed associated with reductions in melanoma

awareness. Indirect evidence of benefit was seen, as the proportion of level I melanomas rose from approximately 9% in the early years of the project (1963–1969) to 26% in 1977. A 25% increase in the proportion of level II melanomas was observed in the same period, and the 5-year proportion of deaths dropped from 41% to 26%. Although melanoma incidence rates in Queensland continued their inexorable rise, mortality rates have stabilized [182]. As noted above, this correlational approach to assessing the benefit of screening is fraught with methodologic hazard. There can be no doubt that these changes are for the better, whatever the explanation.

Similar shifts in melanoma microstage have been reported following large-scale intervention programs in other areas as well. Cristofolini et al. undertook an educational campaign in the Italian province Trento [184]. A particular effort was made to ensure expeditious referral of patients with suspect lesions to free clinics run by the dermatology division of the province's main hospital. Patient consultations increased nearly fourfold as a result of the campaign, excision of melanocytic lesions tripled, and the number of melanomas diagnosed doubled. One new melanoma was detected for every 17 melanocytic lesions excised. The proportion of melanomas <0.76 mm thick rose eightfold from 2.5% before to 21% after the campaign.

In New Mexico, a less systematic educational program, coupled with a very active melanoma tumor registry, was associated with an increase in the proportion of level I/II melanomas from 38% to 61% in a 5-year period [185]. In Austria, a nationwide program was associated with a decline in median tumor thickness from 1.30 mm to 0.95 mm [186]. Of particular importance was the observation that 2 years after the conclusion of a 1-year educational effort, median melanoma thickness had risen back to precampaign levels. This obviously underscores the importance of maintaining the educational effort and screening activity in an ongoing fashion to maximize and sustain the benefit (assuming, of course, that the campaign was truly the cause of the changes observed).

The crown jewel of contemporary large-scale melanoma screening programs is the project currently underway in the west of Scotland. MacKie and coworkers, prompted by the discovery that nearly one third of Scottish patients presented with melanomas >3.5 mm in thickness (5-year survival = 38%), conducted a survey to assess the source of this delay in diagnosis. Their data demonstrated that the delay was attributable to patient's failure to appreciate the significance of their changing pigmented lesion, not to shortcomings in the health care system [197]. Consequently, a public education campaign was designed to inform the public of the features of early melanoma and to encourage them to seek treatment [183]. This project was remarkably thorough in its design and execution.

A concerted and extraordinarily high-quality educational program was constructed for physicians prior to the public phase of the project, as the campaign was based on self-referral of concerned citizens to their family practitioner, who then made the decision regarding referral to a specialist. A

comprehensive, high-quality population-based cancer registry had been in place for an extended period prior to the project, permitting quantitative assessment of the program's impact on melanoma incidence, prognostic features, and mortality. Built into the project were formal studies to quantify changes in workload at institutions doing the testing and changes in the knowledge, attitude, and behavior of physicians and patients. The West of Scotland was the focus of the campaign, and the rest of Scotland served as a comparison.

A sustained increase in the proportion of thin melanomas was observed, with the increase being substantially greater in the West of Scotland, the program's target area. Most exciting of all, MacKie has recently reported a decline in melanoma *mortality* among women in the West of Scotland, the first direct evidence of such an effect ever noted in a melanoma screening program [198]. As this study matures, it promises to provide the best evidence to date in support of the value of melanoma screening.

As described above, the positive predictive value of a screening test can be enhanced greatly if screening is applied to a population in which the prevalence of the disease of interest is higher than it is in the general population. Subgroups of persons at increased risk of melanoma have been described in detail earlier in this discussion. To date, screening has been applied to only one of these groups, i.e., persons with hereditary melanoma and dysplastic nevi.

Greene and colleagues evaluated the development of melanoma in their carefully studied cohort of 14 melanoma-prone kindreds, each of which also displayed dysplastic nevi [14]. Melanomas were stratified into three groups: those that occurred prior to study entry, those that were detected at the initial study examination, and those that developed prospectively during 8 years of subsequent surveillance and regular screening. A substantial shift in melanoma level and thickness (75% of prospective melanomas were <0.76 mm) towards thinner, better prognosis lesions was observed, parallelling the experience cited above in the general population screening programs. These findings have been confirmed and extended by the NCI/Penn team in an expanded cohort that now includes 28 families (MA Tucker, personal communication).

Similar observations have been made by investigators at the University of Pennsylvania [199] and in the Netherlands [200]. The Penn group demonstrated a significant decline from 80% to 41% in the proportion of patients presenting with melanoma in the vertical growth phase (that stage in melanoma tumor progression in which the cancer has acquired the ability to metastasize [3]), documenting that the screening process was associated with (responsible for?) detecting melanoma at a biologically earlier stage in its natural history [199]. None of these surveys have proven a statistically significant decline in melanoma mortality, although the number of deaths among family members with first-time prospectively diagnosed melanomas has been exceedingly low. Of note is the suggestion that clinical photography

may be of particular value in enhancing the efficiency of melanoma surveillance in patients with dysplastic nevi [201,202], although this view is not accepted universally [203]. This technique is not likely to be practical in the general population.

There are several approaches to using high-risk populations to focus screening and education programs. First, one may approach each group of interest separately, designing intervention strategies that are tailored to the needs of each subset. The approach to melanoma/dysplastic nevus family members might well be different than the approach to persons who have had a nonmelanoma skin cancer. Alternatively, one could attempt to develop mathematical models that incorporate multiple risk factors as a way to select from the general population those persons at greatest risk of melanoma. Two such models have been proposed.

MacKie et al. modelled risk factor data from a population-based Scottish case-control study of melanoma and identified total nevus number, freckling, atypical nevi, and number of sunburn episodes as the major determinants of melanoma risk. They then generated a table of melanoma relative risks as a function of the various permutations of these four factors, identifying four strata of melanoma susceptibility [204]. Relative risks ranged from a reference baseline of 1.0 (<20 nevi, no freckles, no atypical nevi, and no sunburn) to a maximum of 173 for women and 587 for men (>20 nevi, freckles present, >2 atypical nevi, and >2 sunburn episodes). They suggest that with the aid of such information, patients could more intelligently self-select themselves for referral to a screening program.

English and Armstrong concluded a similar analysis of their data from the Western Australia melanoma study [205]. Five variables provided maximum discrimination between cases and controls: number of raised nevi on the arms, arrival in Australia before 10 years of age, time spent outdoors in the summer between ages 10 and 24, and family history of melanoma. Their data suggested that 54% of all melanomas arose in an identifiable subset of persons comprising only 16% of the general population. Focusing screening activities on this subset of persons might well yield a favorable cost-benefit outcome in our efforts to reduce melanoma mortality. One drawback to this general approach is the possibility that different populations may demonstrate somewhat different melanoma risk profiles. If true, this would create the onerous burden of conducting substantial epidemiologic studies in each population to be screened. Alternatively, we will need to demonstrate that a model generated in one population can be applied effectively in another, and there is reason to expect that such may be the case.

5. Education

Pending the development of an effective chemoprevention modality for melanoma, the 1992 NIH Consensus Conference correctly pointed out that, 'ongoing public education is the major currently available means of achieving

primary prevention of melanoma' [169]. The two major foci for educational programs are health care providers and the general public. For melanoma risk reduction to succeed, both constituencies must be reached effectively. The importance of professional education cannot be overstated. It would be unfortunate, indeed, for an educated consumer to be greeted by an ill-prepared health care provider; the difficulties encountered by nondermatologists in correctly identifying pigmented skin lesions have already been cited [181], and this report is not exceptional [206,207]. This may be, in part, a consequence of dermatology receiving only 0.24% of total medical school curriculum time [208], and systematic instruction regarding melanoma risk factors is vanishingly rare.

All the major screening programs described above included a major component devoted to professional education [182–186], but the effectiveness of these activites has not been formally assessed. Developing teaching materials that are based on sound educational principles is not a straightforward task, and it seems inefficient for each of the groups around the world with an interest in this issue to 'reinvent the wheel.' Resources should be pooled and a common set of materials should be developed for universal use. From what I have seen of the educational materials developed by MacKie et al. [183], theirs would constitute a superb starting point for such an effort.

The same principle applies to educational materials developed for the general public, although regional variations in lifestyle and tradition still need to be accommodated. In reviewing materials prepared for general consumption, it is not surprising that the same themes recur worldwide: the sun as a melanoma risk factor receives the greatest attention. As a consequence, specific recommendations are tied to reducing sun exposure: (1) Avoid the mid-day sun (a step that reduces UV exposure by 60% [209]), (2) use broad-spectrum sunscreens, (3) seek out the shade on sunny days, (4) wear clothing that physically blocks sunlight from reaching skin (a broad-brimmed hat reduces UV exposure to the face by 70% [210]), (5) avoid sunburn, (6) protect your children from the sun, and (7) know the melanoma warning signs.

With reference to the last item, at least three different sets of criteria have been proposed. Most widely advertised is the 'ABCD' mnemonic proposed by Fitzpatrick et al. [211] and adopted by the AAD: *A*symmetry of shape; *B*order irregularity; *C*olor haphazard (brown, blue, gray, white); and *D*iameter >6 mm. The Glasgow group developed a seven-point checklist to facilitate the identification of suspected melanomas: mild itch; >1 cm diameter; recent growth; irregular edge; irregular pigmentation; inflammation, oozing, and crusting. They suggested that referral for biopsy be made if three or more features were present [212]. Elwood et al. analyzed the first event that led to melanoma diagnosis in 651 patients and reported that 65% displayed one or more signs of a 'classic presentation': enlargement (43%), color change (32%), pain (22%), or bleeding (16%) [213].

Virtually all educational materials alluded to here have been developed for the general public. There is a dearth of materials targetted to specific high-risk populations of the kind outlined earlier in this presentation. If such subgroups are to become a major focus for melanoma intervention activities, such materials will need to be developed.

Educational programs also offer a unique opportunity to gether valuable data regarding patient behavior and attitudes, knowledge of which can be used to design appropriate informational materials or intervention strategies. The example of MacKie and colleagues' recognition of patient ignorance as the main contributor to delay in seeking treatment as the stimulus to their campaign has already been cited [197]. They noted no differences in this regard by gender or by site of melanoma. Others have made similar observations and also have noted that melanomas detected incidentally by physicians who were seeing patients for unrelated problems were, on the average, half as thick as those brought to physicians' attention by their patients [214]. This latter point indicates that one aspect of professional education must be to stress routine skin surveillance as an integral part of ongoing health care.

Hersey et al. compared characteristics of patients with thin (<0.75 mm) and thick (>3.00 mm) primary melanomas among 1300 Australian melanoma patients [215]. They noted that patients with thicker primaries were more likely to be male (68% vs. 45%), be over the age of 50 (75% vs. 33%), display a nodular histology (62% vs. 2%), and have a head/neck primary site (27% vs. 12%). MacKie et al. have reported analogous difficulties in attracting older men into the Glasgow screening program [198]. This subgroup of the general population may require unique strategies to facilitate their participation in screening activities. Workplace-based programs may be of particular value here. Krige et al.'s findings vis-à-vis nodular melanoma are a reminder that not all melanomas originate from precursor lesions that change. Melanoma can arise de novo in clinically normal skin, a fact that is overlooked by the 'melanoma warning sign' approach to recognizing suspicious lesions. The notion that a rapidly growing or new dark nodule (often symmetrical) may herald melanoma may need inclusion on the list of worrisome characteristics.

Girgis et al. performed a random survey of 1344 persons in New South Wales, Australia [216]. They found that 48% of respondents had checked their own skin or had it checked regularly by another, usually a spouse. The notion that we are, in part, responsible for monitoring each other's skin warrants exploration. They also reported that 17% had undergone physician screening in the past year. Persons were less likely to have been screened if they were male, of low socioeconomic status, unemployed, or of primary school education. Market research in Australia has suggested that teenagers value a tan, work to get a tan, believe they look healthier with a tan, perceive a tan as valued by their peers, hold the belief that a tan protects against skin cancer, and those who hold these views tend to be risk seekers

[217]. This is a very difficult set of attitudes to combat, but such data must be taken into account as educational programs for teenagers are developed.

6. Summary

Melanoma is a disease that need not be deadly. Advances in our understanding of the etiology and biology of melanoma over the past 20 years have brought us to the brink of a new era in which the twin goals of primary and secondary prevention may be within our grasp. There is ample reason to be optimistic that this can and will be accomplished.

Note

Since this manuscript was prepared, seveal additional reports of note have appeared in the literature. Cannon-Albright and colleagues have identified another melanoma susceptibility gene in hereditary melanoma families. This gene has been mapped to chromosome 9p13–p22 (218). An interesting new agent with chemoprevention potential has been identified, i.e., a synthetic analogue of alpha-melanotropin (219). Italian invertigators have performed a cost-effectiveness analysis of their melanoma prevention campaign, and estimated program costs to be $400 per year of life saved (220). Mackie and colleagues have published their observations regarding declining melanoma mortality in women following screening (221).

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6. The natural history of melanoma, including the pattern of metastatic spread and the biological basis for metastases — staging of melanoma

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1. Introduction

Melanomas are complex and biologically heterogeneous tumors whose clinical behavior varies from indolent to extremely aggressive. As with any malignancy, it is important to be able to stage melanomas to allow the prediction of clinical behavior for prognosis, treatment determination, and analysis of end results.

2. Evolution of staging for melanoma

Staging for melanoma has evolved rapidly over the last 15 years. The original methods of staging melanoma were based primarily on clinical estimation of disease extent as local (Stage I), regional (Stage II), or systemic (Stage III) [1], as described in Table 1. In this system, Stage I consisted of either an intact primary melanoma, a primary resected within 1 month, or a cutaneous recurrence confined to the initial site of origin. Regional lymph node metastases or intransits were Stage II, and disseminated disease was Stage III. Although there were significant differences in survival between these stages, the majority of patients at initial presentation fell into Stage I, which consisted of a heterogeneous group of primary and locally recurrent tumors.

For this reason, a four-stage system at M.D. Anderson Hospital was devised in which stage I was confined to primary tumors, either intact (Stage IA) or resected (Stage IB); satellite lesions (variously defined as within 3–5 cm of the primary) were Stage II. Stage III consisted of patients with regionally recurrent disease manifested by either: intransit metastases in Stage IIIA (>3–5 cm from the primary tumor, but not beyond first echelon nodes), regional node involvement in Stage IIIB, and intransit + regional node involvement in Stage IIIAB. Stage IV consisted of patients with distant metastases (Table 2). Neither of these systems predicted the behavior of isolated primary melanomas, which constitute roughly 80% of cases at initial presentation, and neither enjoys widespread use at the

L. Nathanson (ed.), Current Research and Clinical Management of Melanoma.

Table 1. Clinical staging system for cutaneous melanoma

Stage I	Localized melanoma without metastasis to distal or regional lymph nodes
	1) Primary melanoma untreated or removed by excisional biopsy within 1 month
	2) Locally metastatic and/or recurrent melanoma
	3) Multiple primary melanomas
Stage II	Metastases confined to regional lymph nodes
	1) Primary melanoma with simultaneous metastases
	2) Primary melanoma controlled with subsequent metastasis
	3) Locally recurrent melanoma with metastasis
	4) Unknown primary with metastasis
Stage III	Disseminated melanoma
	1) Organic and/or multiple lymphatic metastases, and/or
	2) Multiple cutaneous and/or subcutaneous metastases

Table 2. Modified clinical staging system

Stage 1A	Intact primary melanoma
Stage 1B	Primary melanoma resected
Stage II	Satellite metastasis
Stage IIIA	Intransit metastasis
Stage IIIB	Regional node metastasis
Stage IIIAB	Intransit and regional node metastasis
Stage IV	Distant metastasis

present time, though the MDAH staging system is often used by clinicians who perform limb perfusion because of the more extensive lesions with which they deal.

A significant advancement in the understanding of the clinical behavior of melanoma resulted from the use of microscopic methods to stage primary tumors. The first of these methods to be widely used was described by Clark et al. in 1969 [2]. These investigators related prognosis to both the clinical appearance of the primary tumor (superficial spreading, nodular, or lentigo maligna) and to the depth of tumor invasion relative to the anatomic layers of the skin (epidermis, papillary dermis, reticular dermis, and subcutaneous fat), as described in Table 3. Since the skin lymphatics are located in the papillary dermis, invasion of this layer and beyond carried increasing risk of lymphatic and distant dissemination. Soon after, Breslow [3] found that the thickness of the primary melanoma, as measured by an optical micrometer, had a direct relationship to survival. This is generally referred to as microstaging. There is a roughly linear relationship between Clark's level and thickness [4,5], but because the layers of the skin vary in thickness in different individuals and in different anatomic regions, this correlation is not perfect. In practice both Clark's level and Breslow's microstaging are important, as thickness modifies the prognosis within various Clark's level. In unusual clinical variants, such as pedunculated melanomas, which are thick, but often superficially invasive, Breslow's staging provide a more accurate reflection of poor prognosis.

Table 3. Clark's levels

Level I	Tumor remains above the basement membrane (in situ)
Level II	Extension into the papillary dermis
Level III	Extension into the junction of the papillary and reticular dermis
Level IV	Extension into the reticular dermis
Level V	Invasion of the subcutaneous fat

Table 4. Clinico-pathologic staging

Stage IA	Tumor ≤0.75 mm and level II
Stage IB	Tumor >0.75 mm and ≤1.5 mm and/or level III
Stage II	Tumor >1.5 mm and ≤4 mm and/or level IV
Stage IIIA	Tumor >4 mm and/or level V and/or satellites within 2 cm of the primary
Stage IIIB	Metastasis to regional lymph nodes and/or intransit metastasis
Stage IV	Distant metastasis

Using these new pathologic criteria, the AJCC and UICC derived staging criteria based on both clinical and pathological examination (Table 4).

In patients without clinical or radiographic evidence of metastases, the stage as determined by microscopic examination of the primary lesion is one of several important variables (including sex, presence or absence of ulceration, and anatomic location) in making treatment decisions and determining prognosis. Currently, both the size of the wide excision margins and decisions regarding the appropriateness of prophylactic node dissection are influenced by UICC and AJCC tumor stage.

The risk of satellite and intransit metastases has a roughly linear correlation with tumor thickness. Patients with melanomas less than 2.0 mm in thickness have a risk of satellite and intransit metastases of only 2–3%, whereas those with melanomas with a thickness of ≥4.0 have a risk of 5–12%.

Depth and thickness are also related to the risk of lymph node metastases. There is a direct correlation between the level of invasion and the incidence of lymph node metastases [4,6–9]. Indeed, as the level of invasion increases, the proportion of patients presenting with clinically involved nodes increases as well. For thin melanomas less than 1.0 mm, the incidence is very low, averaging no more than about 10%. As the thickness increases to the intermediate range (1.5–4.0 mm), the incidence of lymph node metastases increases from 10% to 25% [4,10], up to 34–57% [10,11]. Performance of an elective node dissection is reported to have an effect on the incidence of lymph node metastases [11,12]. As expected, thick melanomas (>4.00 mm) are associated with the highest incidence of nodal metastases, up to 67%

[11]. The pattern of initial relapse also varies with the type of treatment [13]. In those patients who did not undergo ELND, the regional lymph nodes were the single most common site of first recurrence, whereas for those who did have ELND, nodal relapse as the site of first failure was less than 10%.

In general, the incidence of distant metastases also increases in a roughly linear fashion with the thickness of the primary lesion [10,13]. Skin, subcutaneous tissue, and distant lymph nodes were the most common site of distant metastases in 59% of the patients described by Balch et al. [14]. The most common visceral sites are lung, CNS, liver, and bone [14–16]. Widespread metastases were noted as the initial evidence of distant spread in 4–20% of the patients [14,15].

As the disease progresses clinically, multiple sites are involved in the majority of patients. Prior to death, up to 78% of patients will have multiple sites of involvement [15], especially the skin, lungs, liver, and CNS. CNS metastases are reported as the most common cause of death in one series, followed by metastatic pulmonary disease [17].

Lee [18] reviewed the literature and summarized the patterns of metastatic disease found at autopsy. The other sites that are commonly involved in more advanced disease and that must always be evaluated are the adrenals, kidney, heart, and gastrointestinal tract. Analysis of metastases reveals an apparent clustering with three patterns emerging [19]. One is dominated by the CNS, a second by the lung, and the last by the GI tract.

3. Progress toward biologic staging

The ideal staging system is one that would allow accurate prediction of the risk of subsequent recurrence through the examination of tumor and host properties at the time of initial diagnosis. While clinicopathologically based staging systems, such as AJCC and UICC, represent progress in this direction, much work remains to be done. A predictable number of thin melanomas will be atypically aggressive, and a predictable percentage of thick melanomas will be uncharacteristically benign. Improving our understanding of these variations will require a better understanding of both tumor and host factors that contribute to the processes of local, regional, and systemic metastases in humans.

Over the last 30 years, extensive studies of the metastatic process in inbred animals have contributed greatly to our conceptual framework for understanding the metastatic process in humans. In these animal systems, metastases result from a complex multistep interaction between the tumor and host. Tumor properties contributing to this process are the ability to (1) detach; (2) traverse the interstitial space; (3) invade capillaries and/or lymphatics; (4) evade detection/destruction by local, regional, and systemic components of the immune system, such as tissue macrophages, cytotoxic T

cells, NK cells, cytokines, and antibodies against tumor-associated antigens; (5) invade capillaries in distant organs; (6) adapt to local growth conditions; and (7) reestablish a blood supply [20–25]. One must presume that a system of similar complexity exists in humans. However, several practical problems make it difficult to translate these concepts directly to the biologic staging of human tumors.

In animal systems only a small fraction of cells in unselected tumor may possess a sufficient number of properties to successfully complete the metastatic cascade [20,21]. This suggests that there may be a sampling problem with respect to predicting clinical behavior in human tumors by characterization of a small portion of the overall tumor population.

Secondly, studies in both animals and humans suggest that there may be variations in the level of host immunity as a function of (1) environmental conditions (such as stress, surgery, anesthesia, transfusions); (2) intercurrent diseases (malnutrition, AIDS); (3) treatments (radiation, chemotherapy); and (4) tumor immunosuppressive factors [20,21]. Aside from any set of tumor properties, these variations could well have additional bearing on the risk of metastases in individual patients.

Given both the complexity and variability of these factors, it is unlikely that univariate systems will ever be able to predict metastatic behavior completely unless it is found that expression of the various components of the metastatic process are regulated genetically as a group rather than individually or in subsets [26]. It is also not presently known whether the properties producing tumorifaction (such as uncontrolled cellular growth) and the properties promoting metastases are separately regulated [26].

These caveats not withstanding, we are beginning to accumulate information relevant to the metastatic process in human melanomas. One of the most interesting areas of study is that of the interaction between melanoma cells and other cells as well as the extracellular matrix (ECM). We will focus on these areas.

4. Intercellular interactions and metastases

There is mounting evidence that the expression of class II HLA antigens is altered during the progression of melanoma [27–29]. In one series, class II HLA antigens were found in 50% of the primary melanomas and 100% of the metastases [27]. Further subtyping [29] has shown a difference between the different class II HLA antigens. While HLA-DQ expression was not markedly increased in metastases, those of HLA-DP, and especially HLA-DR, were. Holzman et al. showed that as tumors became progressively thicker, an increasing percentage of cells in the primary lesions were positive for HLA-DR [28]. In lesions $\geqslant 3.0$ mm in thickness, the proportion of cells positive for HLA-DR was essentially the same as it was in metastatic tumors. This increase in HLA-DR expression also correlates with other

markers of advanced lesions, such as high growth fraction as measured by Ki-67 monoclonal antibody [30]. Studies by van Muijen et al. suggest that while increased expression of HLA antigens on tumor cells is associated with disease progression, they may not necessarily play an important role in the actual process of metastases, but may be secondarily increased by the host response to the tumor [31].

Expression of HLA antigens may be induced by the presence of β-IFN in vitro [28,32,33]. The ability of β-IFN to induce these changes in still in question, as different investigators have obtained different results [32,34]. Tumor necrosis factor (TNF) has also been shown to affect HLA expression [35,36], although this may reflect only enhancement and not the de novo appearance of the antigens [36]. The effect of β-IFN may also be operating in metastatic lesions, as evidenced by the relationship between increases in other β-IFN inducible antigens and HLA-DR [29]. Since the pattern of HLA antigen expression may be influenced by host response, it could conceivably be a predictor of survival [37]. That the induction in culture of HLA-DR antigens by IFN was limited to a metastatic variant of a human melanoma cell line also argues for an unknown relationship between the HLA antigenic profile and the metastatic process [32].

Other monoclonal antibodies have also been raised to tumor-associated antigens which show a correlation with progression and metastases [28]. Two of these, P3.58 and MUC18, have created a great deal of interest. Both have been sequenced. P3.58 is identical to the intercellular adhesion molecule I-CAM-1, and MUC18 belongs to the immunoglobulin superfamily and has a sequence similar to the neural cell adhesion molecule (N-CAM) [38,39]. In fact, the whole field of cell adhesion molecules has undergone intense investigation recently, and several aspects seem to be related to the development of metastases [40].

ICAM-1 is a molecule that is usually found in hemopoietic cells. Melanoma is one of the few solid tumors in which it is expressed in a significant percentage of the cases [41]. The exact relationship of this molecule to the process of metastasis is unclear. It is rarely expressed in benign nevi and lentigo maligna [28,39,41]. Expression of ICAM-1 on the cell surface is seen much more often on malignant melanoma. As the Breslow thickness of the tumor increases, there is a corresponding increase in the percentage of cells expressing ICAM-1 [28,39]. According to some studies, the highest level of expression is seen in metastatic lesions, where it may reach 80–90% [39, 41]. Circumstantial evidence for the linkage between ICAM-1, metastases, and survival also comes from Harning et al., who reported that increased serum levels of ICAM-1 were inversely correlated with survival [42]. However, other work has failed to show a perfect correlation between ICAM-1 expression and increasing malignancy. For example, while Hansen et al. [43] discovered the most intense staining for ICAM-1 on metastatic lesions, all benign nevi studied also expressed ICAM-1, and in malignant melanomas there was no correlation between staining and the level of invasion, as has

been shown by other workers. Similarly, studies using human melanoma cell lines failed to show any relationship between metastatic potential in nude mice and levels of ICAM-1 [44].

Other studies have also been done looking at the relationship of other cell adhesion molecules to tumor progression in melanoma. Monoclonal antibody MUC18 recognizes an antigen that is rarely found on normal or dysplastic nevi but is present on 55% of melanomas, 46% of primaries, and 69% of metastatic lesions [45]. There is also a direct correlation between Breslow thickness and Muc 18 antigen expression [39]. A separate study of neural cell adhesion molecules in murine tumors showed decreased expression of N-CAM and the appearance of expression of L2 in cells with the ability to metastasize [46]. Cell adhesion molecules have also been described that mediate the attachment of murine and human melanoma to endothelial cells [47,48].

The field of cell adhesion molecules of the immunoglobulin superfamily was recently reviewed [40], and the conclusion reached was that while CAMs are conceptually attractive as molecules that might be involved in progression and metastases, there is yet no experimental evidence to prove this and it is likely that a complex pattern of changes might be involved. For example, loss of some ICAMs could result in less intracellular adhesion between melanoma cells, which might be important in the process of cell detachment, and increased expression of other ICAMs might result in increased affinity to endothelial cells, which might be important in the process of attachment. As previously discussed, some of these changes might be purely secondary to effects of the host immune system. Indeed, Brocker et al. have reported a distinct change in the composition of the inflammatory infiltrates of primary and metastatic melanomas [49]. The expression of different cytokines has a profound influence on the ICAM-1 expression, which in turn influences the immune response. ICAM-1 expression is increased by interferon β (IFN-β), tumor necrosis factor α (TNF-α), and interleukin-1 (IL1) [33,50,51].

Thus the expression of ICAM-1 is increased by at least two factors (IFN-β, TNF-α), which also affect HLA-DR. These effects occur in vivo, as IFN-β is detectable in human melanoma [49]. In addition, at least some melanoma cell lines are able to produce IL-1 autologously [50].

The expression of HLA class II antigens and ICAM-1 may or may not be involved in the development of metastases, but the presence of both is necessary for the development of immunologic responses to tumors. The absence of ICAM expression is correlated with a relative resistance to cell-mediated lysis [52–54], and pretreatment of resistant cells with cytokines increases HLA expression, ICAM-1 expression, and lysis to a small degree [55]. However, there is no direct correlation between the antiproliferative effect of the cytokines and their effect on antigen expression [33].

5. Extracellular matrix interactions and metastases

The other group of interactions that are involved in the processes of progression and metastasis are those between the tumor cells and the extracellular matrix (ECM) [56]. This includes the adhesive reaction of cell surface receptors with matrix proteins and proteoglycans, as well as changes in the tumor's ability to degrade ECM components.

There are several types of molecules that are involved in the interaction of the tumor cells with the ECM. Both cell surface glycoproteins and glycolipids are involved, as well as receptors and enzymes. The glycoproteins include, but are not limited to, the group of adhesion molecules known as the integrins. Several excellent reviews have recently appeared concerning the alterations in the integrins that are associated with melanoma progression and attachment [57–59]. Integrins are protein heterodimers consisting of one α and one β subunit. The specificity arises from the fact that there are at least 12 different α subunits and at least seven different β subunits [58].

Changes in the distribution of the different subunits have been shown to occur simultaneously with tumor progression [59]. This includes changes in both the β_1 and β_3 subunits. Changes associated with these subunits have been described in studies performed with murine melanomas, human melanomas in nude mice, and human melanoma cell lines. The β_1 subunit is associated with the very late activation (VLA) antigens [60], which were originally described in activated T-cells. They have since been shown to be present on other cell types.

The earliest studies concerning the β_1 subunit were done with the B16 melanoma. In 1989, Kramer et al. [61] reported that attachment to the basement membrane could be blocked by antibodies that reacted with integrin-like receptors on the surface of the B16 cells. This receptor was able to interact specifically with laminin, fibronectin, and type IV collagen. These receptors were further examined and were found to contain the β_1 subunit and three different α subunits [62]. Laminin was bound by an $\alpha_6\beta_1$ complex (VLA-6), which did not bind the other ECM molecules. The complex binding type IV collagen did, however, show some weak binding with laminin. Integrins with the β_1 subunit are also involved in adhesions to fibronectin. Mutants of B16 murine melanoma with decreased metastatic potential had an alteration in the β_1 subunit, which led to a decreased adhesion and spreading on fibronectin and laminin [63].

Parallel studies were also proceeding with human melanoma. The attachment of melanoma cells to collagen types I and IV was mediated by integrins $\alpha_1\beta_1$ (VLA-1) and $\alpha_2\beta_1$ (VLA-2) [51,64]. There is also a marked expression of VLA-2 in a human melanoma cell line that showed a high rate of spontaneous metastases in nude mice, especially compared with non-metastizing cells [65,66]. The interaction of human melanoma cells with fibronectin and laminin is also at least partially dependent on integrins with the β_1 subunit [51]. However, the laminin receptor may involve a novel α

subunit [67]. This $\alpha_7\beta_1$ integrin is present on the majority of melanoma cells, but few other tumors or normal cells, including normal melanocytes [58]. The presence of $\alpha_7\beta_1$ integrin on murine K1735 melanoma cells and its high expression [58] could mark it as the laminin receptor that becomes more prominent in a high metastases variant of K1735 reported by Albini et al. [68]. The ability to invade basement membrane has also been associated with an increased expression of β_1 integrins [52].

Like the molecules involved in intercellular interactions, those involved in interactions with the ECM are also related to the host immune response. The expression of β_1 integrins is sensitive to multiple cytokines, including IL-1, TNF, and γ-IFN [51]. In addition, a high expression of β_1 integrins has been associated with an increased susceptibility to cell-mediated lysis [52].

The other subgroup of integrins that has been associated with melanoma is that containing the β_3 subunit, or what has classically been called the *vitronectin receptor* [57]. It actually recognizes a group of proteins that contain the amino acid sequence Arg-Gly-Asp (RGD), including vitonectrin, fibrinogen, fibronectin, laminin, and thrombospondin. Receptors bearing the β_3 subunit include the glycoproteins IIb and IIIa, which are known to play a role in platelet aggregation.

As previously described for the cell adhesion molecules, there is a documented change in the expression of β_3 integrins during tumor progression. Albeda et al. found that only the vertical growth phase primaries and metastatic melanomas showed the β_3 subunit [69]. A similar relationship was shown between primary and metastatic tumors [51]. This has been expanded further using antibodies to the glycoprotein IIb-IIIa complex [70]. The only tissues other than platelets and endothelial cells that were positive were megakaryocytes, glomelular epithelium, endometrial glands, a rare carcinoma or sarcoma, and 75% of the melanomas. Again, nonneoplastic melanocytes were negative. This glycoprotein may also mediate the interaction between melanoma cells and platelets [71], and thrombospondin [72].

The functional importance of integrins containing the β_3 subunit has been examined in both murine and human melanoma. As far as the importance of the β_3 subunit goes, it seems to be dependent upon the RGD sequence. Peptides containing the RGD sequence have been used in a series of murine experiments. Initial studies involving the injection of RGD peptides with the melanoma cells showed a significant reduction in the formation of pulmonary tumors [73–76]. This effect has special relevance to the formation of metastases, because there was no inhibition of the growth of the primary tumor [74]. This activity is dependent on the exact sequence Arg-Gly-Asp, the number of repeats [74], and the three-dimensional structure [75]. At least part of the effect can be explained by effects on cell adhesion, since these same peptides interfered with melanoma adhesion to fibronectin [74–76] and laminin [76]. In human melanoma the IIb-IIIa glycoprotein may also be involved in all stages of growth. Antibodies against this complex are able to

inhibit the growth of subcutaneously implanted human tumor cells in nude mice [77].

The processes of invasion and endothelial cell adhesion are at least partially related to β_3 integrins. Interleukin-1 has the capacity to augment lung metastases in nude mice [78]. Other studies have shown that IL-1 augmentation of cellular adhesion could be blocked by the addition of RGD peptides [79]. This suggests a receptor-like function of the β_3 integrins, which is consistent with the studies of Seftor et al. [80]. Treatment of cells expressing $\alpha_v\beta_3$ integrin with either antibodies or vitronectin resulted in increased invasion of basement membranes in vitro. This was partially explained by increased type IV collagenase expression.

Although the integrins are involved in binding to ECM, they are obviously not the only receptors. Both fibronectin [81] and thrombospondin [82] have multiple adhesive sites for melanoma, only one of which is sensitive to RGD peptides. In fact, β_3 integrins appear to be related to another membrane structure that also requires calcium, the gangliosides. The disialogangliosides GD2 and GD3 were found to be part of the glycoprotein receptor for vitronectin in human melanoma cells [83] and indeed are able to synergistically increase the binding [84].

The importance of the gangliosides as tumor-associated antigens has been well documented [85]. There are consistently differences in the ganglioside pattern of normal melanocytes and melanomas. In cultures of human melanocytes, the ganglioside GM3 was the major component of the gangliosides. While GM3 is still present in cultured melanoma cells, the absolute level of sialylation is decreased, and there is a marked increase in the relative amount of GD3 present [86]. In addition, GD2, which was not present on melanocytes, is seen at a low level in some melanoma cells. In contrast, immunohistochemical staining of human tissues revealed GM3 in all nevi and the majority of melanomas, both primary and metastatic, but not on normal melanocytes [87]. In this system GD3 was found on normal melanocytes, nevi, and melanoma cells. GD3 was the most frequently expressed ganglioside on metastases. While there was no correlation between thickness and GD2 expression, there was a trend towards a general decrease in ganglioside expression. Of notice was the fact that GD2 was seen in more metastases than primary melanomas. This finding is in agreement with the studies performed by Thurin et al. [88]. They too found GD3 on normal melanocytes, but again found significant levels of GD2 only in advanced primary and metastatic tumors. This was traced at least partially to glycosyltransferase activity, which is not present in normal melanocytes. Another factor that has a modulating effect is the action of cytokines [89]. Interleukin-4 was able to increase the GM3/GD3 ratio and increase immunogenicity. Interleukin-4, interferon, and tumor necrosis factor all increase expression of GD2.

The gangliosides have an important function in modulating cell adhesion.

Incubation of murine melanoma cells with a glucosylceramide synthetase inhibitor resulted in the cells losing their ability to adhere to laminin or type IV collagen [90]. In human melanoma the gangliosides GD2 and GD3 are important in adhesion and in the interaction with ECM proteins. Antibodies to the carbohydrate moiety of gangliosides, including GD3, were able to prevent cell adherence in culture [91]. The interaction of melanoma cells with numerous adhesive proteins, including laminin, collagen, fibronectin, and vitronection, is dependent on GD2 and GD3, and can be blocked by antibodies to these gangliosides [92]. This effect may rely on the electrostatic environment provided by the terminal sialic acid residues. More specific antibodies suggest that the interaction is seen specifically with GD2 [93]. While GD2 and GD3 have been shown to be involved in cell adhesion, GM2 may also have a role in tumor growth [94]. Upon injection of tumor with different ganglioside profiles into nude mice, only the GM2 content correlated with tumor development and growth.

In the glycoproteins as well as the glycolipids, there is evidence of the importance of the carbohydrate structure to function. Antibodies to a specific carbohydrate structure were able to block murine melanoma cell motility and the formation of metastatic tumor in the lungs [95]. This antibody was able to react with 30%–40% of a high-metastatic variant of murine melanoma but few of the low-metastatic variant. The acquisition of a metastasizing phenotype is associated with a decrease in sialic acid, which correlates with a change in the penultimate oligosaccharides and the appearance of a tumor-associated galactosyltransferase [96]. Similar correlations with metastases have been shown with inhibitors of α-mannosidase [97] and the level of fucosylation [98].

As shown with fibronectin [81], there are multiple receptors for the ECM proteins. There is also a 69 kDa cell surface receptor for laminin, which has been well defined [99,100]. Specifically it reacts with a pentapeptide YIGSR on the B1 chain of laminin [101]. Treatment with YIGSR is able to inhibit the formation of lung metastases in B16 murine melanoma, while having no effect on the growth of the primary tumor [102]. The activity of the high-affinity receptor is also important in the binding of human melanoma cells to endothelium [103] and in tumor cell migration [104].

The actual contribution of this receptor in vivo could be more complicated than simply allowing adhesion at a site of invasion. There is an inverse correlation between the level of laminin receptor and the metastatic potential of human melanoma cell lines [105]. In addition, treatment with retinoic acid, which inhibits invasion, results in an increase in the receptor. The presence of a third receptor having galactosyltransferase activity has also been described [106]. The interaction of this cell surface galactosyltransferase with N-linked oligosaccharides on laminin appears necessary for cell spreading.

The attachment of cells to ECM proteins and endothelial cells is important

in the process of metastasis. The interaction of glycoconjugates on the cell surface is an important part of this process. As with other cellular processes, the relationship with other receptors will be of major importance.

Following attachment, the ability of the cells to degrade ECM proteins appears to be crucial to the processes of invasion and development of metastatic deposits. The major proteinases that have been implicated include heparanase, type IV collagenase, and tissue plasminogen activity. Heparanase activity has been defined in metastatic murine melanoma [107]. This activity is directly correlated with the metastatic potential of different subclones [108] and inhibitors, including heparin, which are able to inhibit heparanase activity, can decrease the formation of lung metastases [109,110].

Use of an endogenous heparanase inhibitor gave similar results [111]. The presence of this inhibitor was able to lower ECM degradation and lung metastases by about 60%, while heparin results in about a 30% decrease in ECM degradation. The actual contribution of this endoglucoronidase to basement membrane degradation and invasion in vivo is more difficult to sort out. At least one type of heparanase inhibitor, sulfated chitin, is able to block cellular attachment and invasion of laminin-containing subtrates, in addition to having a significant inhibitory effect on the activity of type IV collagenase [112].

The presence of type IV collagenase activity is also considered important for invasion, since type IV collagen is a major component of basement membranes [56]. The presence of a tumor-associated type IV collagenase has been known since 1979 [113]. Murine and human cell lines with the highest collagenase activity also have the highest rate of spontaneous lung metastases [114,115] and are more invasive in vitro [115]. Experimental modulation of collagenase activity by inhibition or activation results in a corresponding change in invasiveness [116]. Increases in collagenase activity are associated with increased mRNA levels for type IV collagenase [117], suggesting control at the level of transcription. Indeed, upstream sequences have been identified in a highly metastatic cell line and result in a higher level of expression of type IV collagenase [118].

The activity of these upstream sequences can be regulated by reactions at the cell surface. The activity of type IV collagenase can be increased by a portion of the laminin A chain [119], as well as the vitronectin receptor ($\alpha_v\beta_3$) integrin [80], and at least the latter has been shown to act through increases in the mRNA level. In addition, retinoic acid, while decreasing the invasiveness of human melanoma, induces a decrease in the level of type IV collagenase mRNA and activity [105].

The activity of cathepsin B has also been correlated with metastatic potential [120,121]. In addition to direct ECM degradation, it could also act by enhancing the activity of type IV collagenase. A final proteinase activity that has been implicated in metastasis involves the plasminogen activator-plasmin system. The level of tissue type plasminogen activator was increased in concert with collagenase in high metastatic variants [115] and was decreased

in concert with the action of retinoic acid [105]. At least one study, however, found a correlation between invasion and plasmin, instead of between invasion and plasminogen activator [116].

6. Conclusions

1. The staging of melanoma has evolved to more accurately reflect prognosis based on initial clinico-pathologic factors. The major advance in this area has been the advent of microstaging.
2. Progression and metastasis are associated with a number of changes in the way the melanoma cells react with their environment. These include other cells as well as the extracellular matrix (Table 5).
3. These interactions are a two-way street. Cell adhesion molecules, integ-

Table 5. Biology of Metastases

Molecule type	Correlation with tumor stage and/or metastases[a,b]		Consistency of reports	Effect on expression by host-tumor interactions
	Animal	Human		
Class II HLA				
Antigen	Y^b (D)	Y^b (D)		Y
HLA-DQ			N	
HLA-DP		Y^b (D)		
HLA-DR		$Y^{a,b}$ (D)		Y
ICAM-1/P3.58		Y^b (D)	N	Y
MUC18		$Y^{a,b}$ (D)		
NCAM	Y^b (I)			
Integrins				
β1 subunit	Y^b (D)			Y
α1β1				Y
α2β1	Y^b (D)			
α7β1	Y^b (D)			
β3 subunit		$Y^{a,b}$ (D)		
Gangliosides				
GD2 (adhesion)		Y^b (D)		Y
GD3 (adhesion)		Y^b (D)		
GM2 (growth)	Y^a (D)			
Laminin receptor	Y^b (I)			
Heparanase	Y^b (D)			
Type IV collagenase	Y^b (D)			
tissue plasminogen	Y^b (D)		N	

[a] Tumor stage correlation.
[b] Metastasis correlation.

rins, and gangliosides can affect the host-tumor interaction. In return, the expression of these same molecules is affected by these interactions (Table 5).

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7. Intraocular melanomas

Jose A. Sahel and Daniel M. Albert

1. Introduction

Intraocular, or uveal, melanoma is an uncommon malignancy. Yet this tumor, which arises from uveal melanocytes [1–4], represents the most common primary intraocular malignancy, with a high incidence of tumor-related deaths [5,6]. Numerous unanswered questions regarding the etiology, natural history, and appropriate management of this disease continue to stimulate both vivid controversy and active research in ophthalmic oncology. In this chapter we shall deal mainly with melanomas of the choroidal and ciliary body. Iris melanomas are far more benign and pose a less difficult problem in diagnosis and treatment [3].

2. Etiology

Very little is known about the underlying causes of uveal melanomas. Yet several insights have come from various fields, such as epidemiology, genetics, and experimental research, with subsequent emphasis on several risk factors and predisposing conditions.

2.1. Epidemiology

2.1.1. Incidence. Reports from various states or regions of the United States as well as epidemiologic studies from other countries provide generally consistent estimates of the annual age-adjusted incidence rate of uveal melanomas, i.e., 0.6–0.7 per 100,000 population [5–20]. This annual age-adjusted incidence is about one-eighth that of cutaneous melanomas, making uveal melanoma the most common noncutaneous melanoma in the United States [5]. Most data, including the Third National Cancer Survey [10] show that uveal melanomas represent 80% of primary ocular tumors in the adult [6–14]. The tumor arose from the iris in less than 10% of cases [13,16].

2.1.2. Age, race, sex. Although several reports of uveal melanomas in children and teenagers exist [21–28], in most series the median age at diagnosis

L. Nathanson (ed.), Current Research and Clinical Management of Melanoma.

is in the sixth decade, with rates dropping in the eighth decade [13,24,29,30]. Uveal melanomas occur mainly in whites [5,10,14,15,30–33]. The latter have more than eight times higher risk of developing this tumor than blacks and three times higher risk than Asian populations [5,10,14,15,30–33]. The first cases of choroidal melanomas in Native Americans have recently been reported [34].

With the exception of one study [5], uveal melanomas occur with a slight predominance in males [9,16,24,35]. In a series of eyes referred to the Armed Forces Institute of Pathology [36], the ratio of males to females was 6:4.

2.2. *Risk factors*

2.2.1. Predisposing conditions. Despite the profusion of studies on the predisposing roles of congenital melanocytosis, neurofibromatosis, and nevi, there remains a need for unbiased evidence of this [30,37]. Ocular melanocytosis (melanosis oculi) and oculodermal melanocytosis (nevus of Ota) are characterized by congenital unilateral hyperpigmentation of the uveal tract and episclera. In ocular melanocytosis this is associated with increased pigmentation of the conjunctiva and episclera, and in oculodermal melanocytosis of the periorbital skin as well. Several reports link these conditions to an increased risk of uveal melanoma [38–42]. Malignant transformation was stated to occur in 4.6% of reported cases with nevus of Ota. This appears to represent a marked overestimate, since most cases without transformation as well as those occurring in Asians and blacks may be overlooked in this estimate [40].

The presence of iris nevi (Lisch nodules) is well established in neurofibromatosis. The relationship of neurofibromatosis and uveal melanomas, on the other hand, is uncertain [42,43].

Uveal nevi do not appear to increase the risk of posterior uveal melanoma. In 1966, Yanoff and Zimmerman described a nevoid structure at the periphery and along the scleral edge of 73 out of 100 consecutive posterior uveal melanomas examined pathologically. They concluded that most uveal melanomas arise from preexisting nevi, a conclusion supported by several case reports [44,45]. Yet this nevus-like structure has been demonstrated in metastatic as well as experimental choroidal melanomas [45,46]. This configuration can be accounted for by various hypothetical mechanisms, such as compression of tumor cells, confinement by scleral fibers, benign proliferation secondary to the malignancy, or a common oncogenic stimulus [45,46].

A few other studies argue for the association of iris nevi and uveal melanomas [30,45]. In the dysplastic nevus syndrome, an increased risk for uveal melanoma has been postulated but definitive evidence is still lacking [47–50].

Similarly the association of uveal melanomas with other malignancies is under discussion. Turner et al. [51], in a study of 333 consecutive uveal

melanoma patients, showed that (1) risk for non-basal cell cancer was over two times greater than in an age- and sex-matched populations and (2) an association between cutaneous and uveal melanomas was present in three cases. However, Lischko et al. [52], in a case-controlled study (337/800), showed that the association is weak, and Holly et al. [53], in a study of 407 uveal melanoma patients matched geographically with 870 control subjects, found no excess in prior cancer. The related occurrence of bilateral diffuse uveal melanocytic proliferation in patients with systemic malignancy will be discussed below.

2.2.2. Genetics. The familial occurrence of uveal melanomas was first reported by Silcock in 1892 [54] and has been reported several times since then [51,55–57]. A few cytogenetic studies [58–60] argue for the putative role of a recessive oncogene on chromosomes 2 [58] or 3 [59], and showed abnormalities on chromosome 6 and 8 [59,60]. These studies are not conclusive. The application of molecular genetics techniques [61] to the study of this tumor should yield important insights.

2.2.3. Hormones and pregnancy. An increased incidence of ocular melanomas during the child-bearing years [5], and the growth of uveal melanomas during pregnancy was reported in two different studies [62,63]. In a study of female hormones and eye melanoma, Hartge et al. [64] compared 238 melanoma-bearing women with 223 matched control women and demonstrated an increased risk (RR = 1.4) in women with a past history of pregnancy or estrogen substitutive therapy. In contrast, a decreased risk was demonstrated in women who had undergone oophorectomy (RR = 0.6). The interpretation of these findings is speculative but suggests the possibility that tumor growth may be mediated by an increased activity of melanocyte-stimulating hormone [30], increased sunlight sensitivity, or extracellular fluid retention. A recent evaluation, involving a relatively small number of patients, of the influence of reproductive factors on the risk of metastases from uveal melanoma did not demonstrate an adverse effect of pregnancy or oral contraceptives.

2.2.4. Exogenous oncogenic stimuli. Sunlight exposure is currently being investigated as a risk factor for uveal melanoma [66–71]. Tucker et al. described a case-control study of 497 patients with uveal melanoma compared to control subjects (patients with retinal detachment diagnosed during the same 5-year period). This study, despite the limitations related to its retrospective manner and the nature of the control subjects, showed that patients with uveal melanoma had (1) more frequently blue irides (1.7; 95% CL: 1.2–2.5); (2) over 25 freckles (1.4; 95% CL: 1.01–2.0), and (3) some increased sun-exposure habits, such as gardening (1.6; 95% CL: 1.2–2.2). Seddon et al. [67] performed a case-control study of the effect of con-

stitutional factors and UV radiation on 197 New England cases compared with 385 matched population controls and 337 cases from the United States compared with 800 sibling controls. They concluded that personal attributes are strong independent risk factors, including (1) northern European ancestry (6.5; 95% CL: 1.9–22.4), (2) light skin color (3.8; CL: 1.1–12.6), and (3) 10 or more cutaneous nevi (2.8; 95% CL: 1.5–4.9). Some of the evaluated indices of UV exposure associated with a small to moderate increased risk were (1) residence below latitude 40°N (2.8; 95% CL: 1.1–6.9), (2) use of sunlamps (3.4; 95% CL: 1.1–10.3 in the New England study), and (3) intense sun exposure (1.7; 95% CL: 0.9–3.0) [67]. Another team found a four- to sevenfold increased risk by UV exposure and twofold increase in patients with light skin color and skin that became sunburned easily [68].

The role of *occupational, chemical, and drug exposures* was incriminated (1) in a single population of chemical workers, although the role of any particular agent could not be demonstrated [69] and (2) for nicotine in the unusual incidence of melanoma in males [70], although no increased risk for early metastasis was detected in smokers [74]. Several chemicals have been used to induce melanocytic tumors in animals [72–78]. *Viruses* have proven useful to induce animal ocular melanoma models, especially RNA-type viruses such as feline sarcoma virus [79–82]. Moreover, the enzyme reverse transcriptase and virus particles have been demonstrated in uveal melanomas [79–82].

Paraneoplastic uveal melanocytic proliferations have been observed in association with various systemic malignancies, particularly of the ovary and lung [83–85]. These appear as diffuse multinodular infiltrations of the uveal tract by melanocytic cells having predominantly nevoid appearance, although more anaplastic cells have been described. The pathogenesis of this entity remains speculative, possibilities include hamartomatous paraneoplastic proliferation or stimulation of a preexisting tumor [84,85].

2.3. Mortality

As emphasized by Markowitz in a recent review of the mortality from choroidal melanoma, very few reports are sufficiently complete to be informative [86]. The most complete long-term survival studies after enucleation were performed in Denmark by Jensen et al. [16,23] and in Finland by Raivio et al. [29]. In the Finnish study, the 5-, 10-, and 15-year survival rates were, respectively, 65%, 52%, and 46% [29]. In the Danish study, survival rates were similar [23], and at the end of the 25-year period, 51% of patients had died from metastases. Rates of survival after radiation therapy appear comparable in the short-term and mean-term period [87–91]. Yet, as is discussed below, both the validity of these data and the long-term predictability of prognosis after conservative treatment remain a matter of controversy in ophthalmic oncology [92,93]. The median survival of patients with metastatic disease is unequivocally very short, 2–5 months [30,94].

3. Pathology

3.1. Histopathology

The available clinical diagnostic techniques allow for the diagnosis of choroidal melanoma with high accuracy. This, coupled with the increasing use of conservative management methods and the limited acceptance of the safety and usefulness of intraocular biopsy by ophthalmic oncologists, has relegated the importance of histopathology to that of a prognostic tool. Histopathologic study of uveal melanomas remains, nevertheless, an active field of investigation in several major fields, such as immunohistochemistry, morphometry, flow cytometry, cell viability, and radiation effects.

3.1.1. Cytology and classification. The spectrum of variability of pigmented cells composing posterior uveal melanoma was first classified in 1931 by Callender [95] and was modified since by Zimmerman and his coworkers at the Armed Forces Institute of Pathology (AFIP) [3,96–98]. Two major cell types are distinguished: (1) *Spindle cells* have spindle-shaped nuclei and indistinct cell borders. They are cohesive and are often arranged in palisades. In the A subtype, nuclei often show longitudinal folds, nucleoli are not distinct, and mitotic figures are rare. In the B subtype, nuclei are larger, nucleoli are conspicuous, the chromatin is coarse, and mitotic figures are more frequent. (2) *Epithelioid cells* are pleomorphic, poorly cohesive cells with round nuclei, single or multiple large eosinophilic nucleoli, and abundant mitotic figures. Two subtypes are differentiated according to the size of the nuclei and the abundance of the cytoplasm [97–99].

Three tissular types of melanomas can be differentiated according to their cytological characteristics: (1) *Spindle cell melanomas*, composed of spindle cells, represent 30% of uveal melanomas; (2) *epithelioid cell melanomas*, composed of epithelioid cells, represent about 5% of tumors; and (3) *mixed cell tumors* containing both cell types (65%).

Despite its relevance as a major prognostic parameter, this cytologic classification remains too subjective in view of interobserver differences of interpretation [100] and the presence of intermediate cell types [101] in what should rather be considered as a continuous spectrum. Gamel and McLean have proposed several reliable and reproducible cytomorphological methods of objective assessment of uveal melanomas without compromising the prognostic value of the former cytological classification [102–113]. The current best parameters are the inverse standard deviation of the nucleolar areas (ISDNA) and the mean of the largest nucleoli. Marcus et al. have demonstrated that similar information may be obtained by counts of nucleolar organizer regions (NORs) [127].

In spite of the more quantitative nature of ISDNA, the mean of the largest nucleoli, and NORs, the classic histologic classification is still useful in gauging the prognosis [106]. This can be seen in studies of the significance

of a high epithelioid cell content, as demonstrated over the past 40 years by several major works [16,24,31,107–112]. Paul et al. [31] showed that the survival after enucleation was highly dependent of the cell type; at 15 years the survival rates were 85% for spindle A melanomas, 80% for spindle B, 46% for mixed type, and 34% for pure epithelioid tumors. Jensen, in his series of 302 cases followed for 25 years [16,24], showed that less than 1% of spindle A tumors were responsible for metastatic disease, whereas 71% of patients with epithelioid tumors died from metastatic melanoma.

3.1.2. Other methods. Immunohistochemistry has had only limited usefulness in the study of uveal melanomas. S-100 protein and HMB 45 antibodies are only helpful in differentiating uveal melanomas from metastatic tumors [113–116]. These are of little if any value in differentiating melanomas from neural crest tumors or from nevi. Attempts to characterize specific uveal melanoma antigens or antigenic patterns using cutaneous melanoma-specific antibodies have promise, but as yet little clinical usefulness [117–119]. Wang et al. [120] have reported the molecular characterization of a monoclonal antibody (8.1H) shared with cutaneous melanomas showing homologies with transmembrane proteins.

The use of Ki-67 and bromodeoxyuridine labelling as markers of tumor proliferation has not yet provided good correlates with prognosis for life or clinicopathologic parameters [121–123]. This technique may prove useful as a tool to evaluate the effect of preenucleation irradiation [122]. Cell-cycle and flow-cytometry studies [124–126] have showed a good correlation between low cell turnover and spindle cell type [125]. An increased nuclear RNA content was found to be associated with a worse prognosis [126].

These methods need further correlative studies to determine their value in the diagnosis of uveal melanomas [127,128]. More recently, in view of their potential therapeutic interest, *tumor infiltrating lymphocytes* (TIL) in human and animal uveal melanomas have been actively investigated [128–135]. These were detected in 5 among 27 tumors and were analyzed by flow cytometry [128]. T-suppressor: cytotoxic lymphocytes were predominant [128]. Nitta et al. [130] showed a restrictive usage of T-cell receptor genes (V alpha 7) in TIL, which may indicate that these are targeted to a specific tumor antigen. Ksander et al. cultured a TIL line from human choroidal melanoma when adding interleukin-2 to the culture medium. The cultured cells had NK, LAK, and tumor-specific cytotoxic activities [134]. Two recent studies give contradictory results regarding the relationship between the presence of TILs and survival rates [135,136].

The *histopathology of radiation-treated globes* is of value in understanding the mode of action of this technique [137–142]. The aim of treatment, i.e., killing all tumor cells or rendering them incapable of sustained proliferation, is postulated to be achieved through indirect tumor necrosis and hypoxia secondary to blood supply damage [137]. Most studies are biased by the small size of the sample and by the nature of cases examined in the pathology

laboratory. Changes in eyes enucleated for radiation-induced complications or poor tumor control may not reflect the radiation response of most treated cases. Using conventional light microscopy methods, it was difficult in most studies to characterize histopathologically the radiation response aside from radiation-induced damage (radiation retinopathy, rubeosis iridis, cataract, vitreous hemorrhage). Not surprisingly, tumor regrowth is correlated with significant mitotic activity [141], whereas good tumor response is linked with fewer mitotic figures, and tumor and blood vessel damage [142]. It seems likely that loss of replicative capacity through DNA damage is a major mode of action of this method. Other significant features of irradiated tumors include necrosis, fibrosis, and balloon cells [138,140,142].

3.2. Natural history

3.2.1. Doubling time. Over the past 10 years the doubling time of posterior uveal melanomas has been assessed by several authors using various approaches, including inferred natural history from comparison of ages and sizes of tumors at the time of diagnosis [3,143], calculations made from selected reports [144,145], and data provided by the observation of small melanomas prior to enucleation [146–152]. The doubling times of these tumors appears to be extremely variable, ranging from 1 to 2 months to several years, with the growth curve following an exponential pattern, as postulated by Manschot et al. [153].

Spontaneous regression of choroidal melanomas was reported by Lambert et al. [154] and Hardman et al. [155], who retrieved a total of five cases from the literature. Spontaneous regression of experimental melanomas in swine [132] and mice [131] was correlated with a cell-mediated immunological response.

3.2.2. Intraocular growth. Ciliary body and anterior chamber tumors usually grow as ovoid tumors near the equator of the lens, with possible compression and cataract [156–159]. Some tumors, called *ring melanomas*, display a diffuse thickening of the ciliary body. These carry a high metastatic risk. The anterior chamber angle may be affected in several ways. The tumor can extend through the iris root or the scleral spur, displace the iris stroma posteriorly, or obstruct the outflow channels with tumor cells or cell debris either floating free or phagocytized by phagocytic cells [3,156–160]. Secondary glaucoma, called melanomalytic glaucoma, may occur more frequently than in cases of posterior tumors [161–162]. Vitreous seeding is rare with ciliary body melanomas [161].

Choroidal melanomas usually grow initially as nodular, discoid thickening of the choroid. Most assume a 'mushroom' or 'collar button' shape after breaking through Bruch's membrane [3,156–159]. The overlying retinal pigment epithelium is altered morphologically with drusen and several degenerative changes, called *tumor-associated retinal pigment epitheliopathy*.

Lipofucsin accumulation is responsible for the presence of orange pigment on the surface of some melanomas [164]. Retinal detachment, infiltration of the retina, and vitreous seeding occur occasionally [165]. Diffuse choroidal melanomas are often overlooked clinically and are often diagnosed with extraocular extension. They carry a poor prognosis [166].

Infiltration of the sclera by tumor cells along emissary canals and vortex veins is common with large and diffuse melanomas [167].

3.2.3. Extraocular extension. Extraocular extension is generally seen in large or diffuse tumors, but can occasionally occur in small tumors [166–174]. This finding is encountered in about 13% of cases [170–173], and is associated with a high risk for recurrence and metastasis [167,171,173,174]. Extension to the optic nerve and its sheaths may occur in juxtapapillary tumors [175,176].

3.2.4. Metastases. Almost all cases of metastases involve the liver exclusively or in association with other organs, including the lung, gastrointestinal tract, lymph nodes, and the pancreas through hematogenous spread [3,15,177–179]. Patients with preexisting liver disease are more frequently affected [180–181]. The respective role of cell surface properties, nonspecific trapping of tumor cells, and host defenses in the pathogenesis and selectivity of hepatic metastases from uveal melanomas remains to be investigated [182–192].

3.3. Clinical diagnosis

In 1964 Ferry drew considerable attention to the high rate (19%) of enucleation for 'pseudomelanomas,' or lesions misdiagnosed clinically as melanomas [193]. Over the last two decades, a considerably higher level of accuracy has been achieved in the hands of experienced specialists using modern diagnostic techniques, including ultrasonography and fluorescein angiography. Other published data from the AFIP files are self-explanatory: Shields and Zimmerman found that between 1962 and 1969 the rate of misdiagnosis had not improved, whereas it dropped to 6.4% between 1970 and 1980 [194–196]. Data from the Mayo Clinic between 1954 and 1977 give a rate of 2.6% [197]; pathology cases on file at the Ohio State University reveal a rate of 10.9% between 1931 and 1959, which dropped to 1.7% from 1960 to 1981 [198]. Two recent studies from the oncology department at the Wills Eye Hospital [199] and from the multicenter Collaborative Ocular Melanoma Study (COMS) [200] demonstrate an impressively high (over 99%) level of accuracy. Most pseudomelanomas are represented by suspicious choroidal nevi, peripheral disciform degeneration, congenital hypertrophy of the retinal pigment epithelium, choroidal hemangiomas, and metastatic carcinomas [199].

These studies snow that the improvement in diagnostic accuracy is a result of experienced specialists in referral centers combining indirect oph-

thalmoscopy, fundus contact lens examination, scleral transillumination, serial observation, and ancillary techniques, such as ultrasonography, fluorescein angiography, and computed tomography [156–159].

Fluorescein angiography can be helpful in differentiating melanomas from disciform macular degeneration, subretinal hemorrhage, congenital retinal pigment epithelium hypertrophy, and scleritis. It can be more difficult to distinguish and to rule out metastatic carcinoma and choroidal hemangioma by fluorescein angiography [156–159,201–204]. Although no pathognomonic angiographic pattern can be described, the following features appear as the more common in melanoma: early hypofluorescence, progressive staining and late hyperfluorescence, multiple pinpoint leakage foci, and for large melanomas having broken through Bruch's membrane, the double circulation aspect. Retinal pigment epithelial and neuroepithelial alterations are also better demonstrated [201–204].

Ultrasonography, combining standardized A-scan and B-scan, is useful (1) in differentiating pseudomelanomas from melanomas, especially behind opaque media; (2) in determining tumor dimensions; and (3) in detecting extraocular extension [156,157,159,201,205–218]. This information is of value both for planning treatment choice and as prognostic parameters. Typical A-scan patterns include a low or medium internal reflectivity with attenuation between apical and basal high spikes [210,211]. The B-scan pattern comprises the typical discoid or mushroom shape, acoustic hollowness, and choroidal excavation. The reliability of tumor dimension measurements has been questioned in view of (1) a lack of correlation between acoustic (or transillumination) and histologic sizes, and (2) some interobserver variability [206,212,214]. These criticisms do not detract from the role of ultrasonography in this field. Tridimensional reconstructions of the tumor and volume assessments may be attempted [215–218]. Tissue characterization by ultrasonic imaging [213,219] is promising in order to predict tissue type and radiation response. Differentiation of small melanomas elevated less than 2–3 mm from choroidal nevi is difficult and generally requires serial follow-up of such suspicious lesions [150,158,159]. Doppler ultrasound can be useful in determining tumor vascularization and tumor radiation response but requires cautious interpretation [220–224].

Computed tomography provides useful information regarding tumor size and shape, as well as the evaluation of extrascleral extension [225–227]. An impressive experience with magnetic resonance imaging of uveal melanomas has been reported using surface coils, thin sectioning, and gadolinium contrast enhancement [228–237]. This technique reveals the paramagnetic properties of melanin and may become a useful diagnostic tool in the near future.

Immunologic assessment is not yet reliable since no specific monoclonal antibody has yet been identified [238–243]. Radioimmunoscintigraphy using 99 m Tc-labelled monoclonal antibodies is still at a preliminary stage [244,245]. Tumor markers such as carcino-embryonic antigen (CEA) are

used to monitor tumor recurrence or metastasis for some cancers, but their lack of sensitivity in uveal melanomas limits their usefulness [246].

In contrast with many other fields of oncology, fine-needle aspiration biopsy has not yet been accepted as a routine technique in the diagnosis and cytologic typing of uveal melanomas in view of (1) fear for tumor cell seeding [247,248] and (2) difficulties in the interpretation of the aspirates [159,249–251]. This technique, as well as transscleral or transretinal biopsy, is still restricted to the differential diagnosis of difficult cases [247–251]. Without pathology, the evaluation of both prognosis and tumor response to conservative treatment modalities is difficult, resulting in speculative statements and controversies.

The main diagnostic difficulty remains with the differentiation of suspicious nevi from small melanomas. In such difficult cases, observation for evidence of growth is currently the best approach [148,149]. Digital enhanced indocyanine green angiography may improve differentiation [252].

3.4. Prognostic factors

The prognosis of melanomas is dependent on several parameters. The two most significant, using multivariate analysis, are the number of epithelioid cells and the largest tumor diameter [3,99,107–113]. These parameters may become less significant over time after enucleation [257]. Among the many other factors affecting prognosis as determined by univariate analyses, the more significant include ciliary body involvement, the presence of numerous mitotic figures, and extension into the sclera, or worse, beyond the surgical line of transsection [108–114,167,258–260].

Another major factor that has been suspected to be extremely significant is the type of treatment. In the 1970s Zimmerman and McLean speculated that enucleation may increase the risk for metastasis, based on the occurrence of most metastatic deaths in the first 5 years after surgery [3,99,261]. They found speculative arguments for this assumption in the findings by Fraunfelder et al. of tumor cell dissemination during traumatic surgery [262] and in Niederkorn's postulated 'loss of intraocular tumor induced concomitant immunity' [263,264]. These hypotheses have all been challenged [153,258,260,262] in view of the probable link between tumor size upon enucleation and risk for metastasis. The legacy of this once vivid controversy lies in the impetus given to more conservative treatment modalities.

Comparison of survival after enucleation or radiation therapy is still difficult in view of several factors, e.g., lack of histopathologic data for the radiation-treated eyes, shorter follow-up periods for irradiated eyes, and differences in sample types and sizes. Studies reported to date indicate that survival does not apparently differ significantly in the mean term after radiation therapy [263–265], whereas reliable long-term comparisons are not yet available and can only be inferred [265,266]. The results of the prospective Collaborative Ocular Melanoma Study (COMS) undertaken by

the National Eye Institute in 1986 to resolve this issue are therefore awaited by many ocular oncologists [267].

The pattern of tumor regression following radiation is currently considered to be a prognostic indicator, since a rapid decrease of tumor dimensions is correlated with a higher risk for metastasis [268–271]. The interpretation of these data without any histological correlation remains speculative. Tumor regrowth or recurrence after plaque therapy is also a negative factor in reagard to patient survival [272].

4. Therapeutic approaches

In determining the most advisable the various therapeutic options currently available to patients with posterior uveal melanomas, careful evaluation of several factors is necessary. The personal preference of both the physician and patient are of extreme importance in this decision. In addition, it is useful to discuss the management of these tumors in view of the following features: (1) tumor size — small tumors with less than 10 mm diameter and 3 mm elevation, medium size tumors with 10–15 mm diameter and/or 3–5 mm elevation, large tumors with over 15 mm diameter and/or 5 mm elevation, very large melanomas elevated more than 10 mm; (2) status of the opposite eye; (3) visual acuity; (4) location, extension, and growth of the tumor; (5) age, physical, and psychological status of the patient. The COMS group has developed a standard classification for ocular melanomas [273]; none of the other classifications, including the one developed by the American Joint Commission on Cancer on the basis of the TNM system [274], has found wide acceptance. Anecdotal experiences and personal impressions and choices still play a major role in selection of treatment for ocular melanoma. Whereas the necessity and methodology of the COMS are still challenged by some authors on the basis of preexisting retrospective data, the goals set for the ideal treatment by the designers of this study are certainly not disputable: primarily to destroy or totally inactivate the tumor in order to provide the patient with the best life prognosis; and secondarily, to attempt preservation of useful vision in the treated eye.

4.1. Enucleation

For more than a century the classical treatment of posterior uveal melanomas was enucleation. This approach, although still widely supported by some ophthalmologists [265], has lost acceptance over the past 15 years as the standard therapy, especially for small tumors. This has occurred as a consequence of several concurrent events: (1) the high percentage of eyes reported enucleated for pseudomelanomas such as nevi (see above); (2) the reclassification of some spindle A melanomas as nevi [97,98,100]; (3) demonstration by serial observation of untreated small tumors of their relatively

small risk for growth and metastasis [100,143,146,147,153,201,275–278]; (4) progress in techniques of conservative management; and (5) concerns as to the increased risks for metastasis after enucleation (see above) [3,99,153,259–264]. Yet until the results of the COMS become available, which may be as long as 10 years from now, there are valid arguments for enucleation of medium-size and large melanomas. Most authors advise enucleation in patients with very large tumors and a healthy second eye, or if high suspicion of optic nerve invasion or large extraocular extension exists. Failure of conservative treatments is also a good indication of enucleation [158,160].

Gentle or 'minimal' manipulation of the globe is advised during enucleation in order to prevent fluctuations in intraocular pressure [159,279], although the so-called 'no touch technique' [262] is no longer attempted. The motility of the prosthesis is improved by the use of orbital implants, such as integrated hydroxyapatite implants [157,158,280].

The value of preoperative irradiation has been supported by laboratory demonstration of decreased mitotic activity and in vitro viability of melanoma cells after irradiation [280–285]. Clinical evidence of the effectiveness of adjunctive treatment is lacking [286–287]. This question should be clarified when results from this arm of the COMS become available [288].

Edwards and Schachat showed that most enucleated patients (87%) retained a level of visual function comparable to their preenucleation status [289].

4.2. Conservative approaches

4.2.1. Periodic observation. As mentioned above, several authorities have recognized periodic observation of selected melanomas as a valid approach, particularly small- and medium-sized slowly growing melanomas in the patient's only useful eye, especially if the tumor is located close to the optic disk or macula, dormant small melanomas or equivocal cases where documentation of growth is crucial, and where physical or psychological obstacles to other approaches of treatment exist [100,144,147,148,150,154,201,275–277]. This approach requires careful monitoring through repetition of fundus examination, photographs, and ultrasonic evaluation every 3 months [157,159]. If growth is documented, other management strategies need to be considered.

4.2.2. Photocoagulation. This approach was introduced by Meyer-Schwickerath using the xenon arc several decades ago and gained some advocates [290]. The technique has evolved toward the use of argon laser, and in recent years it has been combined with photosensitizers such as hematoporphyrin derivatives and phthalocyanins [291–302]. Yet the high rate of recurrence and complications, coupled with the improvement of

irradiation techniques, has resulted in limited clinical use of this technique [303–305]. The most comprehensive studies were provided by Vogel [292], and Shields et al. [159,304]. These authors report diverging results. In the series of Vogel [292], 54 patients were followed for 20 years. Among these only 46% were considered as cured, 63% were alive, 20% underwent enucleation, and among the 37% who died during follow-up, metastatic death was documented in eight cases. Shields et al., among the 35 patients treated between 1976 and 1979, report no tumor-related deaths and five subsequent enucleations [159]. This technique is usually attempted for small tumors located at more than 3 mm from the fovea, or close to the optic nerve (except temporally or if overhanging the disk margin) [159], in view of the risks of radiation for central vision. Presently, the main use of photocoagulation in ocular oncology is for treatment of serous retinal detachment secondary to choroidal melanoma or even nevi [306].

4.2.3. Radiotherapy of uveal melanoma

4.2.3.1. Radiobiology

4.2.3.1.1. Radiosensitivity of melanoma: Doubling time. Melanoma, and particularly uveal melanoma, is frequently stated to be a radioresistant tumor, presumably because of its high capacity for DNA damage repair [307]. Its degree of differentiation and its long doubling time, ranging from 2 months or less to several years, are at first glance arguments against radiotherapy. Moore, prior to 1940 [308], first attempted radiation treatment of ocular melanomas by implanting radioactive seeds directly within the tumor. For several reasons the development of more sophistocated methods of radiotherapy has continued: (1) the rarity of extraocular spread of small- and medium-size tumors (sclera is thought to be an effective barrier), (2) the disagreement as to the value and risks of surgical techniques, and (3) the lack of effective chemotherapy. Yet data characterizing the radiobiology of uveal melanoma are scarce. A notable example is the fact that the total therapeutic dose is not yet well established. One of the main obstacles to the establishment of accurate radiobiology and dosimetry of human uveal melanomas is the lack of a valid animal model of this tumor. The most commonly used models are either tissue cultures [309,310] or the Greene melanoma [311,312], which may rather be considered as model of animal amelanotic melanoma and therefore not reflect the clinical situation. Other models, either induced in different species [313,314] or spontaneous [315], are available. The growth of uveal melanoma cell lines as xenografts in the rabbit eye may be promising [316]. Until more reliable models are available, both the total dose and fractionation schedule will be learned from both the Greene melanoma model and clinical studies [317,318]. The ongoing challenge remains to cure a radioresistant tumor in a small, radiosensitive organ.

*4.2.3.2. **Ocular radiation sensitivity.*** The germinal zone of the lens epithelium is very sensitive to radiation. Cataracts are generally delayed after irradiation, probably as a consequence of the slow turnover of cells. This complication is rare for doses under 200 cGy, but constant over 750 cGy in a single dose or 1100 cGy delivered during fractionated irradiation [307]. Chronic keratitis can be observed after a dose of 5000 cGy delivered over a 5-week period [307,319,320]. The retinal sensitivity to radiation is similar to brain radiosensitivity. Radiation injury can result from doses of 5000 cGy in 5 weeks. The lacrimal apparatus can usually tolerate doses between 3000 and 4000 cGy with cobalt-60. The optic nerve can develop ischemic injury if the total dose exceeds 6000 cGy or the daily dose exceeds 180–190 cGy [319, 320]. In view of these data, several techniques have been proposed to optimize radiotherapy of uveal melanomas and to minimize the adverse complications.

4.2.4. Types of irradiation

*4.2.4.1. **External radiotherapy.*** External beam radiotherapy with gamma rays produced by cobalt-60 or linear accelerator can be employed before enucleation [281–288] or to treat patients with regional or metastatic extensions [321].

*4.2.4.2. **Brachytherapy.*** Brachytherapy refers to treatment with ionizing radiation applied directly to or a short distance from the body. The rationale of choice of brachytherapy for uveal melanoma is the physical dose distribution nearest the tumor that allows one to theoretically limit the dose delivered to normal tissues surrounding the tumor. The radiobiological efficacy of continuous low-dose irradiation is generally attributed to biologic phenomena such as the repair of sublethal or potentially lethal damage, redistribution, recruitment, and reoxygenation (for dose rates under 100 Cgy/h) [307,322]. In view of the lack of radiobiologic data concerning this tumor, it is difficult at this time to support any hypothesis regarding a preferential action of radiations on tumor cells vs. normal tissues, except for one based on a theoretical modification of cellular kinetics.

Several radionuclides have been used to treat these tumors. Before 1930, Moore implanted a gold-222 radon seed into a cilio-choroidal melanoma [308]. Radon was used more recently at the Ohio State University for a few cases [323]. This radioisotope is derived from radium-226 and its period is 3.82 days. It decays into polonium-218 and lead-214. Many gamma rays and alpha particles of different energy are produced during these decays, and the dosimetry is not easy to perform [324].

In the 1960s Stallard popularized cobalt-60 plaques [325]. This radionuclide has a period of 5.26 years and decays into nickel-60 by emitting a beta particle of 313.3 keV. The nickel is in an excited state and produces two

gamma rays of 1.17 and 1.33 MeV, which are used for therapeutic effects [324]. Since the latter radiations have a long range in tissues, the dosimetry remains relatively crude. Yet the use of such standardized plaques has long been common because of the long half-life of cobalt-60 [87,325–328].

Lommatzsch, followed by several European teams, later published results utilizing episcleral application of ruthenium-106 plaques [88,329–332]. This radionuclide has a period of 368 days and decays into rhodium-106 and palladium-106 by emitting two beta particles, which are used in therapeutics: The first is 39.4 keV, and the second has an energy ranging from 2.07 (1%) to 3.63 MeV (79%), and eventually an additional gamma radiation [324]. The maximum range of the beta rays is 6 mm; and the treatment of tumors thicker than 6 mm is followed by a high level of relapse [138,329–332].

In 1976 Sealy first reported the use of iodine-125 plaques [326]. This radionuclide decays into tellurium-125 by a nucleus electronic capture. The tellurium is released in an excited state and electronic vacancy in the K-bound state, is let into the atom by the electronic capture or by the ejection of an Auger electron, and produces X-ray photons of 27 keV that are used in therapeutics [322,333]. Although this radionuclide has a short period of 60 days, its selection by many as the isotope of choice for brachytherapy of uveal melanoma was largely due to the ease of shielding the adjacent structures by a thin gold coat and the increased safety of medical personnel [335–337]. This isotope has since been chosen as the radiation source for brachytherapy in the COMS [338]. Improvements of the method include better plaque design [339,340], Monte Carlo dosimetry [333,334,341], and tridimensional dosimetry using appropriate treatment planning systems [342].

Preoperative information, including the accurate localization, extension, basal size, and thickness of the tumor, are obtained from clinical examination, A-scan and B-scan ultrasonography, CT scan, or magnetic resonance imaging. Then an appropriate dosimetry is calculated with computer treatment planning systems, e.g., 3-D with projection of the tumor by digitizing a picture of the fundus and of the CT scan [342]. These calculations provide the duration of brachytherapy and the best way to load and orient the seeds in order to optimize the treatment, i.e., to improve the ratio of doses delivered to critical structures and to the tumor.

The prescription dose for iodine-125 vary from author to author. One hundred Gray at the apex of the tumor at a dose rate of 50–125 Cgy/hr is the dose recommended by the COMS [265,343]. Yet, as emphasized earlier, the tumoricidal radiation dose for posterior uveal melanomas is not known and therefore, at best is estimated [344,345].

The plaque is applied surgically under local retrobulbar or general anesthesia. The accurate positioning of the plaque, with a security margin of 2 mm around the tumor, is ensured by transillumination and ophthalmoscopic observation of the tumor, and may eventually be checked by ultrasonography [346,347].

Recently, among new isotopes palladium-103 has been shown to deliver more radiation to the tumor and less dose to normal ocular structures [348].

4.2.4.3. Light ion therapy. Cyclotrons are used to produce proton or helium ion beams of a few microampere of intensity with a tight energy spectrum. Protons of 60 MeV are efficient to treat uveal melanomas, whereas for helium ions an energy four times greater is required [352]. Schematically, cyclotron combines an intense axial magnetic field produced by supra-conductivity and an alternative electric field. The magnetic field is used to guide and to focus the ion beam because of its very strong effect on the charged particle trajectory. As a consequence, ions can reach at the proper time and phase the alternative electric field in radial chambers and be accelerated.

The interest in heavy and charged particle beams lies in the dose distribution at the depth of the tissue and in their ballistic precision, used to attempt protection of radiosensitive adjacent structures. These properties proceed from the well-defined range, the remarkably linear trajectory of the ion beam, and the dose distribution along the axis of penetration that follows the Bragg curve (i.e., with a maximum energy deposition just before stopping) [307,322,349–352]. This is shown by the Bethe formula, which indicates that the stopping power and linear energy transfer (LET) are proportional to the inverse of the square of the speed of the particle. When a proton enters human tissues its speed is high and its energy deposition low: 0.5 keV/μm. Along its range its speed is reduced, with a subsequent increase of its energy deposition in the surrounding tissues up to a maximum of 100 keV/μm. Helium ions reach a maximum of 200 keV/μm [307,351]. Such proton and helium ions are considered to be LET radiation, because the high value that they reach in the peak of Bragg is only located a few micrometers before stopping. The radiobiologic effect (RBE) of such radiation does not increase significantly (1.10–1.15 for protons and 1.30 for alpha particles), and does not show the properties of high LET radiation: a lower oxygen enhancement ratio (OER), a greater effect on tumor with high repair of sublethal damages, and a greater effect on cells in phase G1 or G0 [307,322].

Extremely precise anatomic data are required concerning the size and location of the uveal melanoma to calculate an optimized 3-D dosimetry, which would allow shielding of the critical organs. Surgery is required to localize choroidal melanoma by transillumination and to suture tantalum rings on the sclera around the tumor margins [349–351]. A polystyrene head holder is customized for immobilization of the patient during stimulation and treatment. Orthagonal x-rays are obtained in irradiation conditions, i.e., with the patient fixating a small target at a known angle. The dosimetry and treatment are planned using computer calculation to determine (1) the ideal width of the beam, (2) the spread of the Bragg peak, and (3) the optimal beam angle to irradiate the tumor while minimizing the dose to

critical structures such as the optic disk, macula, lens, and cornea. Saunders et al. [349] recommend the use of a collimator that will place 90% of the isodose curve 2 mm outside the edge of the known tumor volume, and a beam range that places 90% dose point of the Bragg peak 3 mm beyond and 2 mm in front of the tumor. These 2 mm margins are calculated to cover microscopic extension or any error in the treatment plan [349]. The treatment is delivered in one to five fractions over 8 days. The patient is treated in a seated position, rigidly attached with by his or her head holder to a computer-controlled patient positioner. X-rays are made perpendicular to the axis of the beam with the patient in the treatment position, and a second exposure of film is performed with a low dose of radiation, to verify the correct alignment of the beam on the tumor. During the treatment, the movement of the eye is closely monitored with a magnifying closed-circuit television system. A dose of 60–80 Gy (physical dose × the RBE of the ion) is delivered, usually in five fractions [349–353].

4.2.5. Complications. Except for diplopia, following the disinsertion of the rectus muscle for brachytherapy or tantalum ring placement, few early complications are noticed after irradiation of an ocular melanoma. Late complications do occur. They include radiation retinopathy with occlusion of retinal vessels, secondary exudation, vitreous hemorrhage and neovascular glaucoma, radiation papillopathy, and scleral necrosis. Depending on the anterior localization of the tumor, cataracts, punctal occlusion with epiphora, or keratoconjunctivitis sicca can be seen [8,137–142,319,320,354–358].

4.2.6. Results. As discussed above, the prognosis for life is similar after enucleation or radiation therapy, based on the nonrandomized retrospective data available. Tumor regression may occur slowly and follow-up of 2 years or more may be necessary [349–354]. Rapid tumor regression appears to be a poor prognostic feature. Fluorescein angiography and colorcoded Doppler imaging [159] are extremely useful in assessing the tumor response to therapy.

In a prospective series of 100 patients treated with cobalt-60 plaque, Stallard showed 69% of tumor good regression, with a visual acuity ranging from 6/18 to 6/60 in 38 patients and preservation of some useful vision in most patients [325]. In 1985 Markoe and Coll reported that a series of 100 plaque-irradiated patients showed better results than a comparison group of enucleated patients, but only 40% of the irradiated eyes had a visual acuity better than 20/200 at 5 years [87]. Packer et al. observed with iodine-125 a 77.6% survival at 5 years, with 62.1% of these having good vision (i.e., within two Snellen lines of loss) [337]. Long-term results of the first 65 treated patients show 17.2% metastasis, 7.8% local recurrence, and an increasing rate of radiation complications with adverse visual outcome [354]. Lommatzsch showed similar results with ruthenium-106, with a 10-year survival of 67% in 309 cases [88,329,332]. Shields et al. estimated the rate of

poor response at 10% [159] and enucleation in 6% of cases [159]. For proton beam therapy, Gragoudas showed in the first series of 128 patients followed long term, a 5-year survival rate of 80%, with 69% of treated eyes having a visual acuity over 20/200 [89,359]. These authors also estimated the 5-year probability of local tumor control after proton beam therapy at 97% [360]. Sanders has reported 18% complications and 6.7% local failures for the helium ion beam [349].

More definitive data will come from the prospective COMS. The COMS, involving 42 clinical centers and six central units, is conducting two randomized clinical trials. The first compares enucleation vs. brachytherapy with 100 Gy delivered to the tumor apex by iodine-125 plaque. The second tests preoperative external irradiation vs. enucleation alone for large tumors. In 1991, more than 1000 patients were enrolled in this trial [288].

4.2.7. Techniques under investigation

4.2.7.1. Combination of brachytherapy plus hyperthermia. The observation that hyperthermia affected tumors was made in the middle of the last century with the spontaneous regression of cancer patients with fever [361,362]. Cell lethality is proportional to the increase of temperature, suggestive of a biochemical effect. The thermosensitivity is high for cells in the S phase, as well as cells that are hypoxic, poorly fed, or in an acidic medium. All these conditions may be met in the poorly vascularized central portion of tumors; these areas are also of low radiosensitivity [361,362]. A synergistic action can be seen using a combination of radiation plus hyperthermia [322]. Enhancement of the effectiveness of radiotherapy by the adjunctive use of hyperthermia has, therefore, been attempted in order to improve both tumor control and the ratio of the dose to the tumor vs. normal tissues [363–371]. Experimental work has been conducted with the Greene melanoma in the rabbit eye using microwave applicators [364–366,370], ultrasonic applicators [367,369], or localized current field [380] as heating modalities with a good response. Recently patients have been treated by brachytherapy combined with hyperthermia with promising results in terms of local tumor control [368,371]. Experimentally, the combination of hyperthermia with liposome-targeted drug delivery (Bleomycin) has been attempted in rabbits [372].

4.2.7.2. Boron neutron capture therapy (BNCT). BNCT is a twofold therapeutic approach using the combination of selected types of molecules to carry boron-10 preferentially into tumor cells, with subsequent irradiation by a thermal or epithermal neutron [373]. Tissues not targeted by the boron-10 undergo very little damage to the tissues. In contrast, the probability for a boron-10 nucleus to capture a thermal neutron is high (10,000 times more than hydrogen), and the nuclear reaction produces an alpha particle and a lithium ion, carrying energies of 1.47 and 0.85 MeV, respectively. These

high LET (linear energy transfer) particles have a range close to 10 μm, which is the size of a cell [373–375]. Ideally, selective incorporation of boron-10 into tumor cells could allow selective therapy. This technique was first suggested by G.L. Locker in 1936, and the first clinical trial took place in the 1950s and 1960s in patients with high-grade gliomas. This trial was not successful because of the use of nonselective boron carriers and thermal neutrons, which are reduced twice with every 1.8 cm of tissue depth [373]. The excellent results of H. Hatanaka [376], however, on high-grade gliomas with another molecule, borosulphhydril (BSH), and of Y. Mishima [377,378] on cutaneous melanoma with a precursor of melanin, the p-10-boro-L-phenylalanine (BPA), has given renewed hope for future applications to cutaneous or uveal melanomas as well as for metastatic disease.

Packer and Codere have shown the preferential uptake of BPA on Greene melanoma grown in the anterior chamber of a rabbit eye and later the cure of this tumor with BNCT [379–380]. The uptake of BPA in human uveal melanoma as well as the application to different models are under investigation [381].

4.2.8. Local resection. Local resection of uveal tumors was promoted by Foulds [382,383], Reese and Jones [384], and several others [385–391]. This technique has evolved over the past 15 years and, in the hands of highly experienced surgeons, appears as good an alternative to irradiation or enucleation in selected case [392,393]. Yet most authors agree that this technique, which induces a higher rate of initial complications, should be considered as an alternative to enucleation only when other forms of conservative therapy are not suitable [391,395]. Techniques include iridocyclectomy for anterior tumors, full-thickness eyewall resection, and partial lamellar sclerouvectomy [385–393]. This last technique, which appears to be safer surgically, has given rise to concerns regarding residual intrascleral melanoma. A local resection may leave substantial tumor cells [396].

Immunotherapy and chemotherapy have not proven useful in local tumor control [397–401]. Such approaches may become useful in the treatment of subclinical metastases, which are likely to be present in many cases at the time of diagnosis.

5. Management of extraocular extension and metastasis

5.1. Extraocular extension

It is essential to detect extraocular extension of uveal melanomas since (1) its existence is an adverse prognostic feature [173–175] and (2) appropriate management must be planned. Preoperatively, ultrasonography or CT and MRI can be very informative. At the time of enucleation or plaque place-

ment, careful observation of the sclera during surgery is mandatory, with frozen sections if necessary. Upon pathologic examination, serial sections with careful examination of emissary canals and vortex vein lumen are essential. Most authors are reluctant to advise radical surgery in such cases because of the high mortality rates observed either after enucleation (two thirds of 60 patients in the series of Affeldt et al. [402]) or exenteration [403]. Some authors [159,404] attempt radiation therapy with either plaques for nodules thinner than 2 mm or a proton beam [404] when extraocular extension is observed.

A maximal surgical approach has been challenged in view of the lack of improvement of life prognosis [170,403–407]. Some authors recommend a modified enucleation technique of en bloc excision of the scleral nodule, the adjacent Tenon's capsule, and eventually the adjacent orbital tissues [159]. Postenucleation orbital radiotherapy is advocated [321].

5.2. Systemic metastasis

The several chemotherapeutic and/or immunotherapy protocols available for the treatment of metastatic uveal melanoma are essentially palliative. A few reports mention successful local resection of solitary hepatic metastasis from uveal melanoma [408,409]. However, widespread metastases are very likely to occur in such patients [159]. Extended lengths of survival after hepatic artery chemoembolization with polyvinyl sponge and cisplatin have been reported [410–412].

The exact place of adjuvant chemotherapy and immunotherapy have will probably be learned from research in the management of metastatic cutaneous melanoma [413–416]. Yet recent experimental studies show some promise regarding the efficacy of dacarbazine (DTIC [417]) and difluoromethylornithine [418] in the prevention, and of LS2616 [419] or BNCT [420] in the treatment, of metastases from murine ocular melanoma.

To date, metastatic melanoma is almost incurable. Avoidance of this condition is the first responsibility prior to the preservation of sight in the management of every patient presenting with uveal melanoma, as well as the goal of continuing research in ophthalmic oncology.

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8. Molecular genetics of human malignant melanoma

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1. Introduction

The idea that cancer is a genetic disease is over 80 years old [1]; that cancer is also a progressive genetic disease is a more recent, but fundamentally critical, expansion of this idea [2]. Due to increasingly sensitive techniques and novel methodological advancements, the supposition that tumors form because of a progressive development in the number and types of genetic defects is being confirmed at the molecular level [3]. Molecular geneticists are now faced with two formidable tasks. The first is to decipher the precise nature and sequence of genetic perturbations that characterize the malignant process at each of its progressive stages. The second is to define the biochemical and biological impact of these genetic events on the interdependent mechanisms that govern the proliferation, differentiation, and intercellular relationships of the normal cell. In this regard, human malignant melanoma provides a particularly good model for studying progressive etiologically relevant events, since clinical and pathological observations have defined cutaneous lesions that represent sequential steps in the progression to melanoma [4–6]. Moreover, cells representing these stages (e.g., normal melanocytes, dysplastic nevi, primary and metastatic melanomas) can be cultured in sufficient quantities to permit a wide range of experiments, including the development of in vitro models of transformation. Consistent abnormalities in chromosomes 1, 6, 7, and 9, as well as alterations in antigen expression, biological characteristics, differentiation programs, growth factor requirements, and proto-oncogene expression and extinction, have been observed to accompany tumor progression of the melanocyte [7–10]. A wide range of studies have begun to define the biological importance of these alterations. In this article we will review the molecular genetics of human malignant melanoma. The subjects touched upon will include (1) correlations between DNA ploidy and prognosis, (2) chromosome 9p deletions, (3) chromosome 1p abnormalities (both in sporadic and familial cases of melanoma), (4) the role of chromosome 6q, (5) the potential role of other chromosomes (e.g., 2, 3, 7, 11, and 10), (6) the involvement of known oncogenes, (7) the molecular analysis of genes on targeted chromosomes,

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(8) development of a model for melanoma progression, (9) the consequences of ultraviolet radiation, and (10) the overall impact of these studies on the clinical management of the patient with melanoma.

2. Biology of human malignant melanoma

The incidence of malignant melanoma (MM) of the skin is undergoing a dramatic increase in persons with light-colored skin in all parts of the world [11,12]. In the United States in 1935 only 1 in 1500 persons developed MM, whereas by the year 2000 it is estimated that 1 in 100 will develop MM. It is not only an increase in diagnosis, since total deaths from MM have also been increasing despite the improved patient survival associated with early surgical removal of lesions [13,14]. The increase in MM presents dermatologists, pathologists, and basic scientists with an unprecedented challenge and an opportunity to explore a number of important questions for which we have no satisfactory answers. For example, how can the clinical recognition of early, and therefore curable, melanoma be improved so that its detection is maximized while unnecessary surgical treatment of other pigmented lesions that lack malignant potential is minimized? What is the relationship, etiologically and diagnostically, of melanoma to potential precursor lesions, such as common acquired nevi, atypical nevi, and congenital nevi? What is the optimal surgical management of melanoma with regard to adequate margins of resection and to the possible benefits of excising clinically uninvolved lymph nodes? How do environmental factors, specifically ultraviolet radiation (UVR), influence the development of melanoma? Finally, what are the specific molecular defects that initiate and cause the progression of MM? To answer these questions, new investigative tools are required. The present clinical and even histologic criteria for diagnosis of melanoma are imperfect, and thus the management of controversial cases remains in dispute. Furthermore, even among unequivocal cases of melanoma, there are variations in the biologic behavior that confound our present ability to predict such crucial events as the risk of metastasis. This suggests that even histopathologically similar lesions are heterogeneous at the molecular and cellular levels. Such heterogeneity is probably related to differences in the genetic events controlling transformation of melanocytes in different cases of melanoma. Moreover, the continuing controversy surrounding the identification and association of atypical 'dysplastic' nevi as an increased risk factor for developing MM belies the need for distinguishing markers other than architecture and cytology. If the nature and temporal sequence of these genetic defects could be documented, it might be possible to tailor therapy to particular cases more efficiently. Thus, the types of studies outlined in this review hold the promise of addressing crucial questions of tumor biology in a natural model system that is uniquely accessible to observation.

3. Ploidy of malignant melanomas

Based on histological, statistical, antigenic, and clinical analyses, as well as in vitro model systems, there are at least five steps in the progression of the diploid human melanocyte to an aneuploid metastatic melanoma cell [4–6]. These are shown schematically in Figure 1. In this review we will consider the progression to a junctional and compound nevus with atypia as a single step, but in fact it may be two sequential steps in the transformation of the melanocyte. In the model shown in Figure 1, we presume that during tumor progression there is an accumulation of genetic defects, and that UVR, genotype, time, and host response probably play crucial roles in the ultimate development of the metastatic melanoma cell.

A wealth of information over the past 10 years has documented the accumulation of genetic and biological defects accompanying the melanocyte through this sequence. Clinicians have attempted to use several of these parameters to predict the clinical course of MM, including Breslow thickness [15], Clark level [16], histopathologic parameters [17], and DNA ploidy [18]. In particular, the use of DNA ploidy of tumors as a predictor of outcome has gained wide acceptance. This is due to belief in one of the fundamental tenets of tumor biology, which is that cancer is characterized by aneuploidy and chromosomal aberrations, and that these genetic alterations are significant events [19].

Consequently, it has become dogma that aneuploid tumors from all types of neoplasms usually give a worse prognosis than near-diploid tumors [20–23]. Similarly, tumors with a disproportionately high number of cells traversing the S-phase of the cell cycle also have a worse prognosis than tumors in which the S-phase fraction is similar to normal tissue [24]. However, while numerous studies have found a clear correlation of aneuploidy with poor prognosis, there are many exceptions to this bias. In MM, a number of studies have analyzed DNA ploidy and S-phase fraction as independent predictors of clinical outcome [18,24,25]. While MM shows a great degree of variability in DNA content and the ability to metastasize and recur, DNA

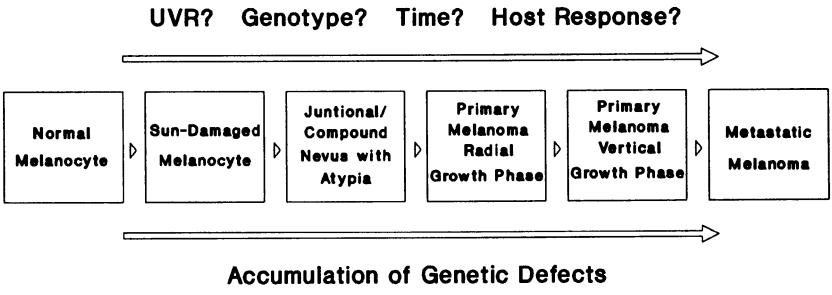

Figure 1. Hypothesized stages in the malignant transformation of the human melanocyte.

aneuploidy, as determined by flow cytometry, is a significant poor prognostic factor [26–31]. There are, however, studies that do not fully agree with this position [25,26,32,33].

Two recent papers point up the confusing nature of using DNA ploidy measurements for predicting clinical outcome. In one of these studies, a novel technique was used in which single nuclei of tumor cells from fresh-frozen tissues of 30 patients with either primary or metastatic melanoma were analyzed [18]. This technique has the advantage of being able to measure the DNA content of a relatively small number of tumor nuclei (as compared to flow cytometry, which needs a larger number of cells) and may be the method of choice for analyzing thin primary melanomas in the radial growth phase. Using this technique, 79% of primary MM and 75% of locally recurrent and distant MM were aneuploid. Patient follow-up (averaging 5.8 years) showed that all patients with euploid primary melanomas showed no evidence of recurrence or metastasis, whereas 4 of 11 patients with aneuploid primary melanomas had local recurrence or distant metastases, and two of these patients died at 6 and 27 months. Of the patients whose metastases were analyzed, 3 of 4 patients with euploid tumors were free of disease, as opposed to only 3 of 12 patients with aneuploid metastases. This study demonstrated, once again, that both primary and metastatic melanomas have an equal probability of containing both diploid and aneuploid cell populations, and that aneuploidy is a good prognostic indicator of poor clinical outcome. However, it also showed that even high risk melanomas can have a euploid DNA content.

In the second much larger study, the prognostic value of DNA content in both fresh and paraffin-embedded melanoma metastases from 95 patients was measured by flow cytometry [24]. This study showed that of the 95 specimens, 33% were diploid and 67% were aneuploid. Moreover, euploid tumors had a significantly lower percentage of cycling cells in S-phase than did aneuploid tumors and were correlated with longer survival. Surprisingly, however, patients with euploid tumors had a worse prognosis (median survival of 16 months) after the first tumor recurrence than did patients with aneuploid tumors (median survival of 28 months). The conclusion of this study that DNA aneuploidy is a favorable clinical feature is in direct contrast to a number of other studies [26–31].

The most tenable explanation to explain these conflicting results could well reside in the form of treatment that the patients received. In the latter study [24], patients with inoperable tumors were given systemic chemotherapy, which changed sequentially as patients failed the preceding regimen. These regimens were utilized regardless of DNA ploidy. The sequence of chemotherapeutic protocols involved cis-platinum plus etoposide, alpha-interferon, and a four-drug combination with interferon. Consequently, it could simply be that these chemotherapeutic drugs impacted more on aneuploid than diploid tumors, thus contributing to a longer survival for the patients with aneuploid tumors.

Another recent study has determined whether the independent parameters of DNA content and elevated proliferation indices could identify atypical nevi that had undergone early steps of malignant transformation but that had not yet undergone morphological atypia [34]. This study analyzed 18 intradermal nevi, 40 atypical 'dysplastic' nevi, and 16 superficial malignant melanomas (<0.76 mm in depth). All of the atypical nevi were of the compound type and were selected using the criteria of Elder [35]. Flow cytometry detected no significant differences between the proliferation indices of intradermal or atypical nevi. However, the proliferation indices for the superficial primary melanomas were significantly higher than those of the atypical nevi. More important, aneuploidy was not demonstrated in any of the intradermal or atypical nevi, whereas 3 of 12 primary melanomas were aneuploid.

These data suggest that atypical 'dysplastic' nevi can be distinguished from early primary melanomas by differences in proliferative indices in which the S, G_2, and M phases of cell cycle are combined and by the absence of any DNA aneuploidy. Moreover, these data suggest that the types of genetic abnormalities involved in the early phases of malignant transformation in a majority of primary melanomas do not grossly perturb the cytogenetic architecture. If these results can be confirmed using a larger series of samples, DNA aneuploidy and proliferative indices could be used to differentiate atypical nevi from early primary melanomas. While there are some contrasting studies [26,36], the absence of aneuploidy in atypical nevi is a consistent finding [27,28,37–39]. Thus, these data support the still controversial view that atypical 'dysplastic' nevi are morphological (but perhaps not biological) variants of normal acquired nevi and are, therefore, unlikely to be a significant source of premalignant melanoma cells [40].

At present, current perceptions concerning DNA ploidy and clinical outcome can be summarized as follows: (1) a low S-phase fraction of cycling cells in any lesion (e.g., atypical nevus, primary or metastatic melanoma) is a good prognostic indicator for survival; (2) aneuploidy, regardless of whether or not the S-phase fraction is greater than normal cells, is a clear indicator of malignancy and should serve to differentiate primary melanomas from 'dysplastic' and mormal compound nevi; (3) even high-risk melanomas can be diploid or near-diploid; (4) depending on the treatment protocols, aneuploid tumors may respond better and thereby lead to a good prognosis for long-term survival; (5) even thin superficial spreading malignant melanomas (less than 0.75 mm) may have progressed genetically and become aneuploid with a predictably worse clinical course; and (6) most atypical 'dysplastic' nevi may not be true progenitors of malignant melanomas but may be examples of architectural polymorphism in which the morphology may appear aberrant but actually reflects a less common form of a common acquired nevus.

At the chromosomal level, these studies point up the genetic heterogeneity of MM and only serve to stress the conclusion that early markers of trans-

formation would be invaluable. Why do some diploid metastases pose a greater risk than some aneuploid tumors? Disregarding host responses, which are difficult to assess at the present time [41], it may be because the aneuploid tumor, despite having numerical and structural aberrations, does not have one or more crucial defects necessary for the development of a tumor manifesting poor prognosis [23]. Clearly, genetic aberrations that are seen in early lesions, retained during progression, or present in a majority of MM warrant scrutiny as being those with critical importance to the transformation process.

It would be easy to argue, however, that the vast majority of cytogenetic abnormalities seen in most solid tumors are unnecessary for the critical phases of a tumor's life cycle and merely accompany the malignant process, offering, perhaps, only a slight selective advantage. This supposition is born out in the treatment of certain tumor types, e.g., central nervous system tumors, bladder carcinomas, etc., in which aneuploid tumors are more sensitive to chemotherapy or radiation therapy than diploid tumors [42]. In CNS tumors, especially, it is not uncommon to treat an aneuploid primary brain tumor, only to have a recurrence that has a near-diploid DNA content, but that has as poor a prognosis as the aneuploid progenitor [43,44]. The observation that aneuploid tumors can have a better prognosis [24] could be useful clinically if shown to correlate with specific forms of chemotherapeutic modalities. Knowledge of the specific types of DNA defects (e.g., ploidy, chromosomal markers, etc.) that may be good or bad prognostic indicators may present opportunities to tailor a patient's treatment.

4. Chromosomal abnormalities

Numerous studies have implicated a role in carcinogenesis for dominant-acting oncogenes, activated by mutation, chromosomal rearrangements, insertion of a nearby promotor element, gene amplification, and enhanced transcription [45]. Other studies indicate that tumorigenicity behaves also as a recessive trait, in that dominant genetic elements (tumor suppressor genes) must first be inactivated in various ways, such as gene or chromosomal deletion, rearrangement, or mutation, in order for the cell to become neoplastic [46]. Presumably, normal progenitor cells require a specific series of gene activations and inactivations to complete the transformation process. Tumor induction and progression is associated with DNA recombination, as evidenced by the fact that many tumors carry aberrant chromosomes generated by intrachromosomal or interchromosomal rearrangements, such as deletions, inversions, or translocations [47,48]. These chromosomal rearrangements are frequently tumor specific and occur in the vicinity of cellular protooncogenes or tumor suppressor genes that are presumed to be involved in oncogenesis [45]. In other cases, the rearrangements appear to be random and their involvement in tumor induction and progression is uncertain.

At present, current efforts to identify specific genes involved in the pathogenesis of MM have relied on two assumptions. First, etiologically significant genes reside at or near chromosomal regions that are frequently rearranged in melanoma and/or show genetic linkage in families presenting with dysplastic nevus syndrome (DNS) or otherwise predisposed to melanoma. Second, a good deal of genetic damage is not revealed by cytogenetic or loss of heterozygosity (LOH) analysis, as the defects involve alterations in gene expression or biochemical functions. These assumptions have precipitated a large amount of research that has attempted to (1) localize the chromosomal regions where putative genes involved in the etiology of MM reside, and (2) detect contributing roles for alterations in the structure, expression, and function of oncogenes, tumor suppressor genes, growth factors, cytokines, transcription factors, and adhesion molecules in the development of MM.

Cytogenetics, familial linkage studies, LOH analyses, and fusions between normal and malignant cells have all contributed significantly to these efforts and have resulted in the identification of a number of chromosomal regions presumed to harbor 'melanoma-associated' oncogenes or tumor suppressor loci. These studies are consistent in supporting the conclusion that genes on chromosomes 1, 6, 7, and 9, and, to a lesser extent, genes on chromosomes 2, 3, 10, and 11, are involved in the pathogenesis of MM [7,10,23,49–54].

Cytogenetic analyses have been especially crucial in pinpointing the genomic regions frequently disrupted in MM. A compilation of cytogenetic reports from over 30 independent studies, dating from 1973 to 1991, indicates that rearrangements of 1p, 6p, 7, and 9p occur most frequently in MM, with anomalies involving chromosomes 2, 3, 10, and 11 also being observed in a significant proportion of the same tumors (Table 1) [53,55–58]. In the case of 1p, 6q, and 9p, consistent losses or partial deletions of these chromosomes (as opposed to additional copies or recurrent translocations), predict that the putative 'melanoma' genes on these chromosomes are tumor suppressors or downregulators of normal cell growth. The cloning of the first two tumor suppressor loci, namely, the retinoblastoma (*RB1*) and Wilms' tumor (*WT1*) genes, stemmed directly from cytogenetic observations of analogous types of rearrangements involving chromosomes 13 and 11, respectively, in these pediatric neoplasms [59–63]. In contrast, frequent polyploidy of chromosome 7 in MM is presumed to result in the overexpression of a growth-promoting gene or oncogene [64].

Molecular analyses, including genetic linkage and LOH studies, have aided in further defining and narrowing chromosomal regions identified as harboring genes involved in melanoma development. Approximately 8–12% of all cases of cutaneous melanoma are inherited in a familial setting [65]. Individuals at risk can be identified due to an overabundance of atypical 'dysplastic', as well as normal nevi, and are often diagnosed as having dysplastic nevus syndrome (DNS) [66]. DNS is inherited as a highly penetrant autosomal dominant disorder with underlying predisposition to MM [67]. Genetic linkage studies have been performed on families showing either

Table 1. Autosomal aberrations in malignant melanoma[a]

Chromosome	Approx. frequency[b]	Region[c]	Timing[d]
1	82%	1p11-p22(q11-q32)	Late
6	64%	6q11-q27	Intermediate/late
7	61%	7p22-q11	Late
9	46%	9p24-q12	Early
11	39%	11p11-p24(p11-q23)	Intermediate/late
3	38%	2p23-q21	Late
2	32%	2pter-q11	Late
14	27%[f]	ND[e]	ND
10	26%	10q11-q26	Early
8	26%	ND	ND
5	25%	ND	ND
15	25%[f]	ND	ND
13	23%	ND	ND
17	22%	ND	ND
21	21%	ND	ND
12	20%	ND	ND
4	19%	ND	ND
16	18%	ND	ND
20	13%	ND	ND
19	12%	ND	ND
22	10%	ND	ND
18	7%	ND	ND

[a] Revised from Fountain et al. [53] with the incorporation of additional cytogenetic data [55,57,58,297].

[b] Frequency of involvement was determined by assessing all chromosomal rearrangements or deletions in each tumor. In the case of chromosome 7, an increase in copy number was also noted. Final percentage values represent the review of 97 or more melanomas.

[c] Regions that show the highest frequency of rearrangements.

[d] The time of chromosomal alteration during melanoma progression was determined primarily on the basis of cytogenetic findings in benign and atypical 'dysplastic' nevi, in addition to data generated on sets of related tumors (see ref. 53 for further discussion).

[e] ND = not determined.

[f] May be artifactually high due to the inclusion of melanoma cell lines that were generated after tumor inoculation into nu/nu mice [57,58]. Rearrangements of chromosomes 14 and 15 were observed infrequently in tumors cultured solely by in vitro means.

inheritance of DNS/MM or MM in the absence of DNS. In one of the first studies, Bale et al. [68] followed up an original weak linkage of DNS/MM to chromosome 1p [69] and reported that there was a high likelihood that a 1p gene was critical for the development of DNS/MM in their families. Odds of over 1000:1 in favor of linkage to 1p were obtained and reflected a maximum lod score of 3.62 occurring at the 1p34-36 marker D1S47 [68,70]. Four subsequent studies, however, have failed to confirm this linkage [71–74]. Possible explanations for these disparate findings include genetic heter-

ogeneity between familial melanoma families and/or differences in diagnostic criteria between the studies.

LOH analyses, involving the comparison of constitutional and melanoma DNA genotypes, have been performed to further highlight specific regions of interest on chromosomes 1, 6, 7, and 9, and to identify any other genomic regions, previously unmasked by cytogenetic studies, in which additional 'melanoma' tumor suppressor loci may reside. In this type of analysis, loci that are constitutionally heterozygous are informative and hemizygous losses of alleles in a number of unrelated melanomas are presumed to mark the locations of tumor suppressor genes [75]. The detection of complete or homozygous losses (seen in much smaller proportions than hemizygous losses) has subsequently been quite instrumental in pinpointing the exact region in which to look for these putative genes. The *RB1*, *WT1*, and *DCC* tumor suppressor genes were all rapidly localized and cloned due to the discovery of homozygous deletions of all or a portion of these genes in crucial tumors [60,63,76]. Overall, in melanoma the LOH data support the presence of critical tumor suppressor genes on 1p, 6q, 9p, and, though somewhat more tentative, on 10q [53,77–81].

In the case of 6q and 9p, somatic cell hybrid data also confirm the presence of growth-inhibiting genes on these chromosomes [82–87]. It has recently been shown that introduction of a normal copy of chromosome 6 into melanoma cells can cause a reversal in some transformation-related phenotypic characteristics and in tumorigenic potential [87]. Analogously, the introduction of chromosome 9 into mouse L cells is also postulated to suppress cell growth [85], and additional studies indicate that a region on mouse chromosome 4 (which is homologous to 9p in humans [88–90]) is a strong suppressor of malignancy [82,83].

Determining the precise temporal sequence of events in melanoma progression will ultimately be critical for designing future diagnostic and treatment strategies. One of the most important outcomes of both cytogenetic and molecular analyses of MM has been the ability to predict the timing of specific genetic perturbations during tumor progression [53]. Studies of precursor (i.e., benign and or atypical 'dysplastic' nevi) and primary lesions are obviously invaluable in determining and ordering the earliest events that occur during melanoma development. The availability of these tissues for research studies, however, is often quite limited, and only a small number of studies have been reported [8,9,52,91]. As a consequence, several recent studies have relied on the analysis of multiple tumors (usually metastases or a primary and a series of metastases) derived from individual patients to predict the timing of events during melanoma progression [7,10,49,78,81,92–94]. In these cases, identical genetic alterations detected in all members of an autologous set of metastases are thought to result from a single event that occurred in the original precursor or primary lesion. The consistent targeting of the same chromosomal region or locus in two or more independent sets of multiple metastases is then presumed to point to an

event that may play an initiating role in the development of all melanomas. Analogously, events that occur in only a subset of related tumors are thought to represent late perturbations (occurring after the development of metastatic potential) and most likely aid in the growth of the established tumor, but not in tumor initiation. Details will be presented here on the chromosomal regions presumed to harbor 'melanoma-associated' genes and, where possible, the timing of mutations within these regions will also be discussed.

4.1. Chromosome 1

Cytogenetic rearrangements of chromosome 1 are detected in approximately 82% of all melanomas and, as mentioned above, genetic linkage studies also indicate that a region on the terminal portion of 1p harbors a gene responsible for at least some forms of familial melanoma [53,68]. LOH studies also support the involvement of a tumor suppressor gene located within this region, since 60% of metastatic melanomas show reduction on 1p [79]. It remains unclear, however, whether there is only one 'melanoma' gene on chromosome 1. There is some evidence to suggest that the familial locus is a separate entity with an as yet unknown function [79]. Furthermore, both LOH and cytogenetic data indicate that the 1p tumor suppressor gene is involved in late melanoma tumor progression, since alterations of 1p are frequently noted in metastatic tumors but are rarely seen in precursor or primary lesions [53,79]. This fact alone, however, does not eliminate the potential involvement of the 1p familial locus, since in an analogous setting germline *p53* mutations have been found in Li-Fraumeni families, while somatic mutations of the *p53* gene occur more often in late stages of tumor development [95,96]. Thus, the 1p familial locus and the 1p tumor suppressor gene could potentially be the same gene.

Two genes that reside on distal 1p, TCL5 and $p58^{clk-1}$, have recently been identified as potential candidates for playing roles in melanoma tumor progression [97,98]. TCL5 was originally cloned due to a translocation between 1p32 and 14q11 in a T-lymphoblastic leukemia [97]. Though postulated to have oncogenic potential (rather than tumor suppressor functions), a rearrangement of the TCL5 gene has been detected in a melanoma cell line [97]. Whether TCL5 is over or underexpressed in melanomas, however, remains to be investigated. Further analysis of the expression of this gene is warranted, since a fragile site on 1p32 is overexpressed in lymphocytes from melanoma patients when compared to those from normal individuals [99]. The possibility exists that the expression of a gene within this region (e.g., TCL5) is affected by this phenomenon and somehow leads to an enhancement of tumorigenic potential. In contrast, the $p58^{clk-1}$ protein kinase gene resides further telomeric, on 1p36, and codes for a cell division-control related protein [98]. Functional analyses suggest that $p58^{clk-1}$ behaves as a suppressor of cell growth and, therefore, makes an attractive candidate gene for involve-

ment in melanoma [98,100,101] (in addition to other neural crest derived tumors that frequently exhibit alterations in this region of 1p). This gene is also located within the region that shows the strongest linkage in the familial melanoma cases mapped to 1p [68].

4.2. Chromosome 6

Chromosome 6 is rearranged in approximately 64% of all MM, and both LOH and somatic cell hybrid studies indicate a 6q tumor suppressor gene is involved in the development of melanoma [23,53,80]. Approximately 40–50% LOH is detected on 6q in metastatic melanomas, with the region most frequently targeted being 6q22-q27 [80]. The c-*myb* oncogene maps within this region (on 6q22) [102], and rearrangements of this locus (or neighboring DNA) have been detected in two independent studies [103,104]. However, c-*myb* alterations appear to be rare events in melanoma overall, suggesting that the ones detected to date arose coincidentally within the vicinity of this growth-promoting gene. All other evidence indicates that the critical locus on this chromosome has tumor suppressive, not oncogenic, activity. As discussed previously, microcell fusion of a normal chromosome 6 into melanoma cell lines diminishes, not enhances, tumorigenic potential [23]. In addition, the relatively high level of LOH on 6q is clearly more indicative of the involvement of a tumor suppressor gene than an oncogene.

Thought somewhat more controversial than the evidence for chromosome 1, perturbation of chromosome 6 also appears to be a late event in this disease. Precursor and primary tumors, as well as multiple specimens from single patients, do not consistently harbor cytogenetic rearrangements or deletions of 6q [9,10,49,52,78,94]. These findings suggest that the 6q tumor suppressor gene is not involved in the early stages of melanoma progression but may contribute more to the enhancement of tumor growth or invasion.

4.3. Chromosome 7

As opposed to chromosomes 1 and 6, frequent polyploidy of chromosome 7 (in 61% of all melanomas) suggests that the putative locus on this chromosome is an oncogene or promotor of cell growth. The accumulation of additional copies of this chromosome during late stages of melanoma progression is well documented and, as postulated, has been correlated with the over-expression of the epidermal growth factor receptor (*EGFr*) [64], a gene involved in the enhancement of proliferation. Though the *EGFr* maps to 7p12-13 [105], the presence of extra copies of 7q in some melanomas, in the absence of 7p, indicates that an unrelated oncogene on chromosome 7 may also be crucial to melanoma development. The recent localization of invasion and metastasis genes to this chromosome adds further credence to this hypothesis [106].

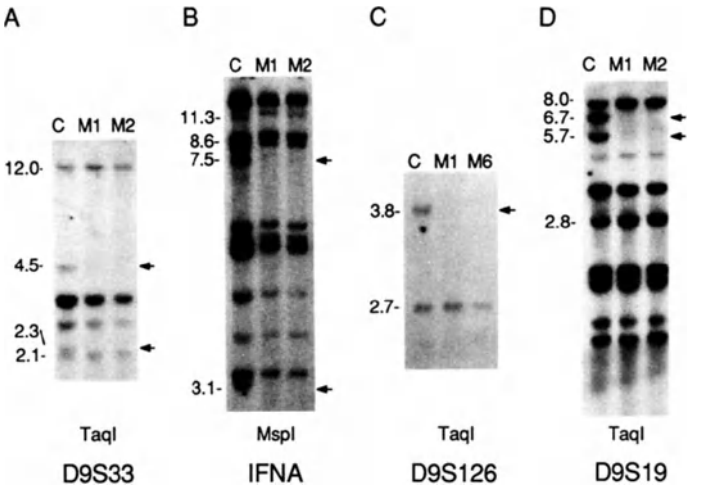

Figure 2. LOH analysis on 9p showing identical allele losses in two (out of six total) autologous metastases from patient DX. Control lymphoblastoid (C) and metastatic melanoma (M1, M2, and M6) cell line DNAs were digested to completion with the indicated restriction enzymes, blotted, and hybridized with probes that recognize the loci (from 9p23 to 9p21) D9S33, IFNA, D9S126, and D9S19 [81]. In all four cases, loss of the same alleles (see arrows) was observed in the six related tumor DNAs, two of which are represented in each panel.

4.4. Chromosome 9

A number of recent genetic studies have focused on the involvement of chromosome 9 in MM [9,10,58,78,81,94]. Though cytogenetic loss or rearrangement of chromosome 9 occurs in only 46% of all melanomas, LOH on 9p is much higher (on the order of 86%) than that seen on either 1p or 6q [81]. The detection of homozygous DNA deletions in two unrelated melanomas has also resulted in rapidly narrowing the critical region implicated on chromosome 9 to 2–3 Mb within 9p21. In addition, chromosome 9 rearrangements and/or identical allele losses have been demonstrated in multiple independent metastases from individual patients (for example, see Fig. 2) [10,78,81,94], and chromosome 9 losses or rearrangements have been detected in dysplastic nevi [9,52]. These findings suggest that the presumptive gene in this region plays an early or initiating role in melanoma progression. The identification of this specific 'melanoma' gene could thus be crucial in developing more effective diagnosis and treatment strategies for MM.

A number of potential candidate genes reside within this region of 9p and include the β-interferon locus (*IFNB1*), the α-interferon (*IFNA*) gene cluster, the melanoma differentiation antigen *TYRP*, and the glycosylation enzyme *GGTB2* [107–112]. The interferon genes make especially attractive candidates for involvement in MM, since they clearly function as anti-proliferative

agents. Interferon-like molecules have also been detected in the basal layer of the epidermis [113], and the administration of α-interferon causes the regression of metastatic melanoma in some patients [114,115]. The *TYRP* melanoma differentiation antigen is a member of the tyrosinase-related gene family [116] and already has a defined role in the development of melanoma [117]. Lastly, *GGTB2* encodes a glycosylation enzyme, and defects in this protein could potentially lead to altered cell morphology or cell growth [118,119]. Though these genes may play some contributing role in the pathogenesis of melanoma, all of them have been eliminated as the 9p 'melanoma' gene, since they reside outside of the limits of the homozygous deletions [81]. Current efforts are aimed at identifying other candidate genes in this region.

The involvement of this same region of 9p or homologous regions on mouse and rat chromosomes has been well documented in a variety of human and rodent malignancies [82,120–128]. Some of the most compelling evidence comes from mouse studies, where a region on mouse chromosome 4 (homologous to human 9p) has been shown to be capable of suppressing the malignant phenotype in mouse melanoma [82]. This finding suggests that the etiology of melanoma may be similar between mouse and human, and indicates that the mouse could offer an appropriate model in which to study the effects of this gene. In this regard, the normal function of the pertinent mouse gene has already been partially elucidated [84,86]. Mouse melanoma hybrids that show suppression of malignancy (i.e., they contain a normal chromosome 4) synthesize ninefold higher quantities of type 1 procollagen, retain more fibronectin in the cell, and release less proteases into the medium than their unsuppressed counterparts [86]. These phenotypic alterations are clearly associated with the retention of the normal chromosome 4 in the hybrids and are thought to be markers of terminal differentiation. It is postulated that a gene on chromosome 4 controls the expression of a myriad of other genes involved in differentiation [84,86] and could conceivably be a transcription factor or a cellular protein with complex protein or RNA interactions.

4.5. Chromosomes 2, 3, 10, and 11

A gene on chromosome 10q may also play an early role in melanoma development. Two independent studies on dysplastic nevi have revealed rearrangements involving 10q24-q26, and 67% of metastatic melanomas show LOH in this region [77,8,52]. Rearrangements of chromosomes 2, 3, and 11 have also been detected in 32–39% of MM. The less-frequent involvement of these chromosomes may reflect molecular heterogeneity, whereby some tumors harbor mutations of genes on these chromosomes and others do not. If this is case, it is somewhat more difficult at this time to predict the overall timing of these events in melanoma. However, current cytogenetic data indicate that the genes on chromosomes 2 and 3 function

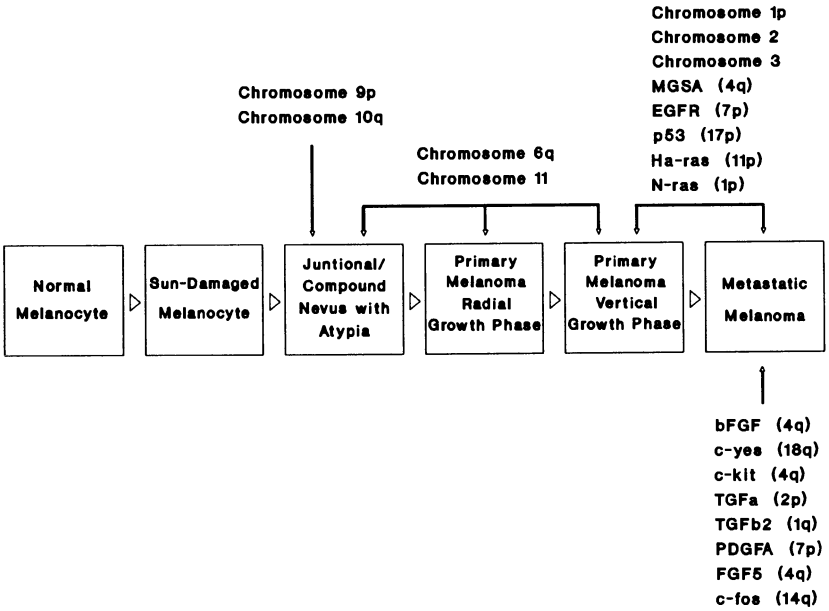

Figure 3. Proposed model of tumor progression from melanocyte to metastatic melanoma. The proposed sequence of genetic events accompanying tumor progression is based on the results of karyotypic and molecular analyses of nevi and melanomas (see text for details).

late in tumor progression, while the putative locus on chromosome 11 may act earlier [see ref. 53 for further discussion].

Though the ordering of chromosomal alterations can be determined with some accuracy (Fig. 3), the combination of critical events in any one specific tumor or type of melanoma remains to be elucidated. Genes on chromosomes 1, 6, and 9, however, appear to play universal roles in most MM, with chromosomes 1 and 6 exerting effects late in tumor progression and chromosome 9 having an early, possibly initiating, role. Other genetic alterations, such as those involving genes on chromosomes 2, 3, 10, and 11, may prove to be heterogeneous, but could still be critical in predicting the clinical outcome in patients with MM.

5. Identification of defects in known genes

Complementing the types of investigations detailed above are studies designed to define both the specific types of damage to known genes and the biological results of this damage. To date, the two most consistently perturbed genes found in human cancers are the *ras* proto-oncogene gene family and the *p53* tumor suppressor gene [129,130]. These genes play very

different roles in the biology of cells. The *ras* family of genes, Ha-*ras*, Ki-*ras*, and N-*ras*, function as GTP/GDP binding proteins with intrinsic GTPase activity [131]. Almost every fetal and adult tissue expresses the *ras* encoded p21 protein, which has fundamental roles in signal transduction, cellular proliferation, and terminal differentiation [131]. All three genes have four coding domains, and point mutations in the first and second exons endow the normal p21 protein with transforming potential. In contrast, the *p53* gene appears to act as a negative regulator of cell growth. The inhibitory effects of the wild-type protein can be inactivated by point mutations, deletions, and insertion mutations. Analysis of neoplasias derived from breast, lung, brain, colon, or mesenchyme tissues has detailed the clustering of *p53* gene mutations in five highly mutable areas, which account for 73% of the observed mutations detected in the coding region of the *p53* gene in human neoplasias and coincide precisely with the four most highly conserved regions of the *p53* gene [130,132]. These data suggest that these mutations play an important role in mediating the abnormal functioning of the *p53* protein. In some tumors, mutations in both *ras* and *p53* genes may be complementary and may be involved in the full expression of the malignant phenotype [133].

5.1. ras genes and MM

The three functional members of the *ras* gene family reside on human chromosome 12p (Ki-*ras*), chromosome 11p (Ha-*ras*), and chromosome 1p (N-*ras*). In several studies [134–137], we have examined over 100 noncultured and cultured melanomas (including invasive primary melanomas and metastatic melanomas) and over 75 related precursor specimens (e.g., in situ primary melanomas, atypical 'dysplastic' nevi, and ordinary benign nevi) for (1) the presence of mutated *ras* genes, (2) correlation of mutated *ras* genes with differentiation-related characteristics, (3) expression of *ras*-encoded p21 proteins, (4) quantitative expression of mutated and wild-type *ras*-encoded p21 proteins, (5) correlation of expression of both mutated and normal p21 with cell cycle kinetics, and (6) correlation of *ras* mutations with the site of the tumor and clinical stage. In addition, we have introduced mutationally activated *ras* oncogenes into normal diploid human melanocytes and gauged the resulting effects. These studies have allowed the following conclusions.

First, the precise role of *ras* oncogenes in the pathogenesis of melanoma remains complex [138]. This is due to the fact that, though the majority of melanocytic tumors do not contain mutated *ras* genes, mutations in this family of protooncogenes remain the most common specific gene defect in both primary and metastatic melanomas. The results of numerous studies [93,129,134,139–144] demonstrated that the N-*ras* gene is (1) the predominant *ras* gene activated in MM and (2) preferentially mutated in the second coding exon at the 61st codon. Results from our studies are shown in Table 2. The frequency of *ras* point mutations is approximately 24% in cultured

Table 2. Frequency of *ras* mutations in melanomas and precursor lesions

Specimens	Number of samples	Oncogene activated	Frequency	Position of mutation	Substituted amino acid
Cultured specimens					
Metastatic melanomas	55	N-*ras*/ Ha-*ras*	24%/3%	61 or 13	lys/arg/leu
Noncultured specimens					
Metastatic melanomas	25	N-*ras*	12%	61	Lys
Primary melanomas	19	N-*ras*	12%	61	Lys
Atypical 'dysplastic' nevi	30	None	0%	—	—
Normal nevi	15	None	0%	—	—

Table 3. Quantitative analysis of abnormal melanocytes in paraffin sections

Specimen diagnosis	Mean percentage of abnormal melanocytes (range)	Range percentage of all melanocytes
Benign nevi	0	20–75
Atypical 'dysplastic' nevi		
Minimal dysplasia	20 (2.5–42)	12–49
Moderate dysplasia	21 (7.6–37)	14–69
Severe dysplasia	15 (11–20)	18–54
Malignant melanoma, in situ	22 (11–28)	11–28
Malignant melanoma, invasive	64 (34–93)	34–93

Abnormal: Junctional melanocytes in the region of intraepidermal proliferation in atypical 'dysplastic' nevi and in situ melanomas, but includes melanoma cells in the dermis in invasive lesions.
Melanocytes: Intraepidermal melanocytes, intraepidermal and dermal nevus cells, or melanoma cells.
Atypical nevi: 'Dysplastic' nevi were graded on the extent of the architectural distortion and on the degree of cytologic atypism of junctional melanocytes.

metastatic melanomas, and 12% in noncultured primary and metastatic melanomas, with greater than 90% of these point mutations occurring at codon 61 of the N-*ras* gene.

The significance of this relative specificity is unclear. One possibility, discussed below, may be that UVR preferentially targets the N-*ras* gene in vivo. Mutations in the Ha-*ras* gene were infrequently detected in MM, and mutations in the Ki-*ras* gene were never detected. In our study, we quantitated the percentage of abnormal melanocytes in the noncultured sections (Table 3). This type of quantitation is extremely important when analyzing melanocytic specimens contaminated with other cell types in order to be sure that the percentage of melanocytes is adequate for the limits of the technique being used. In this case, the ability to detect a point mutation in a *ras* gene

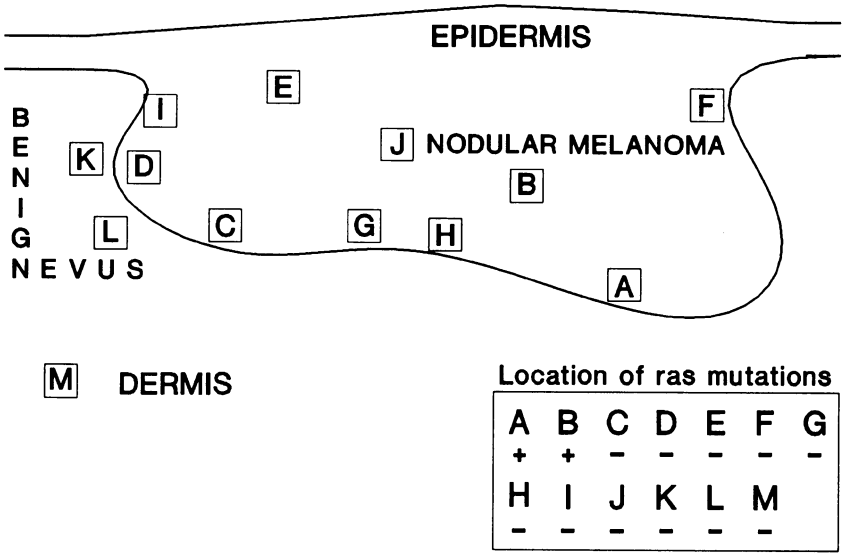

Figure 4. Schematic of a nodular melanoma, surrounding a benign nevus, and dermis showing locations that were microdissected and analyzed for the presence of mutated *ras* genes. The chart on the bottom shows the location of mutated *ras* alleles.

using polymerase chain methodology (PCR) and oligonucleotide hybridization requires that the mutation be present in at least 2% of the total number of cells present [134]. In each of our specimens, the mean percentage of abnormal melanocytes ranged from 2.5% to 93%.

Second, no specimen of nevi or atypical 'dysplastic' nevi had a detectable *ras* gene mutation. Thus, it is possible now to pinpoint accurately when *ras* gene mutations are generated during the development of melanoma and to assume that *ras* gene mutations are not involved in the development of atypical nevi but occur at a later stage of progression. This raises the secondary question of whether *ras* mutations are initiating events or secondary events in the transformation process. Our analysis detected mutated *ras* genes only in invasive primary melanomas in a dermal (vertical) growth phase and not in any early primary melanoma (i.e., melanoma in situ and radial growth phase superficial spreading melanoma). These data suggested that *ras* mutations were not an initiating step in the development of the primary melanoma.

We have investigated this further by a microdissection study in which selected areas (each representing 125 cell equivalents) of primary melanoma specimens were analyzed for the presence of mutated *ras* genes. Figure 4 shows a schematic representation of a primary nodular melanoma from which DNA was extracted, PCR amplified, and analyzed for *ras* mutations. Only the tumor area represented by portions designated A and B contained

an N-*ras* point mutation at the 61st codon. Since the mutation was not detected in all areas of this lesion, these data would argue that *ras* gene mutations are a late event, occurring subsequent to the events that generate the primary melanoma cell. Furthermore, there is evidence that suggests *ras* mutations may not be involved in the generation of the metastatic melanoma cell either. For example, there is no increase in *ras* mutation frequency upon acquisition of the metastatic phenotype [134,139], and only 1 of 6 independent metastases from an individual patient had a mutated *ras* gene [93].

Our inability to detect mutations in atypical 'dysplastic' nevi could be expected, since epidemiologic studies stress that the majority of sporadic melanomas do not arise in association with an identifiable precursor lesion [4,145]. However, a recent study has reported that mutations in the Ki-*ras* gene were not only the most frequently mutated *ras* gene in primary and metastatic melanomas, but were also found in 25% of atypical 'dysplastic' nevi [140]. As discussed above, mutations in any of the coding exons of the Ki-*ras* gene were never detected in any melanocytic lesion in a large number of studies. Possible explanations for this discrepancy have been discussed [137]. However, if future studies confirm the presence of Ki-*ras* mutations in both premalignant and malignant melanocytic lesions, it may suggest that Ki-*ras* mutations are an early, and possibly initiating, event in a subset of MM. One caveat to this speculation, however, is that if in fact 25% of atypical nevi harbor a Ki-*ras* mutation, it is evident from epidemiological studies that the vast majority of these cells do not progress to clinically definable melanoma [146]. Thus, the accumulated data have failed, as yet, to answer the important question of whether *ras* mutations are initiating events or secondary events.

Third, we have also analyzed whether the inappropriate or enhanced expression of *ras* p21 protein correlates with transformation [134]. Immunoperoxidase assays were performed on a large series of melanocytic lesions using crossreactive anti-p21 mouse monoclonal antibodies (Mab) [147]. There were no detectable quantitative or qualitative alterations in the expression of *ras* p21 proteins between normal nevi, and primary or metastatic melanomas, regardless of whether or not they contained a mutated *ras* gene. It appears, therefore, that increased and/or idiopathic expression of p21 is not required for the maintenance, metastasis, or cellular proliferation of MM. This interpretation was supported by a flow cytometric analysis of metastatic melanoma cell lines that correlated the specific binding of an anti-p21 Mab with cell cycle kinetics and the presence or absence of a mutated *ras* gene [134]. This experiment allowed the following conclusions: (1) The amount of p21 expressed is not related to the different growth phases and cell cycle phases of the melanoma cell lines studied, (2) there is no differences in p21 expression detectable between melanoma cell lines containing a mutated *ras* oncogene or a normal *ras* proto-oncogene, and (3) the presence of *ras* mutations did not result in differences in cell cycle kinetics when compared to melanoma cell lines without *ras* mutations.

Fourth, *ras* oncogenes may impact more on the mechanisms that control differentiation than malignant transformation. We base this conclusion on the fact that all melanoma cell lines with *ras* point mutations had a number of phenotypic characteristics in common, which placed the cells in a similar stage of differentiation in the melanocytic lineage. It has been shown that antigenic phenotype can identify melanomas that are representative of early, intermediate, or late stages of melanocyte differentiation [117,148]. A similar analysis determined that the antigenic characteristics of melanomas that contained mutated *ras* oncogenes were remarkably similar, in that each line showed (1) high expression of EGFr and Class II major histocompatibility antigens, (2) a lack of pigmentation, and (3) an epithelioid/spindle type of morphology. These features are characteristic of early stages of melanocyte differentiation. In contrast, the majority of melanomas that did not have mutated *ras* genes displayed antigenic and biological phenotypes representative of the intermediate and late stages of melanocyte differentiation. Presumably, *ras* mutations initially occur in mature, well-differentiated melanocytes that have a biological and antigenic phenotype characteristic of the late stage of differentiation. The activation of *ras* genes by point mutation may somehow induce specific 'dedifferentiation', resulting in a melanoma cell that possesses features characteristic of earlier phases of melanocyte development. These observations as well as others [131] suggest that mutated *ras* genes may, in addition to their obvious transforming potential, have an impact on the cellular controls that affect differentiation. The biological and clinical significance of differentiation-related effects of *ras* oncogenes remain obscure.

Finally, the activation of *ras* oncogenes in less than 25% of primary and metastatic melanomas suggests that these genes do not have a principal involvement in the pathogenesis of most MM. Moreover, despite some conflicting results, the N-*ras* gene appears to be the preferentially mutated and, thus, activated family member. Though melanomas have a high frequency of somatically induced hemizygosity at a large number of alleles on many different chromosomes [78], there is no evidence of a nonrandom hemizygosity/homozygosity in the N-*ras* gene [79]. In addition, linkage data suggest the presence of a melanoma susceptibility locus on the short arm of chromosome 1 [69], but the location of N-*ras* (1p13) is well proximal to 1p34-36 and, therefore, eliminates it as a contender [68,70]. Thus, the N-*ras* gene is not the sought after tumor suppressor and/or familial melanoma locus on 1p.

6. Model systems of melanocyte transformation

Although the *ras* protooncogene family has been implicated in basic cellular processes, such as signal transduction, cellular proliferation, and terminal differentiation [131], the precise types of perturbations induced by *ras* that result in cell transformation are unknown. One possible effect of *ras* oncogene

expression in normal diploid cells may be the generation of chromosomal instability [149–151]. This could result in a range of genetic alterations, some of which would be lethal or neutral, while others would have a positive impact on growth and proliferation. Genetic instability could eventually result in a biological advantage, such as release from controls of the local tissue milieu and increased proliferative capacity, along with a potential for biologic and genetic progression, thus driving the cell towards a transformed and, ultimately neoplastic, phenotype. This scenario is all the more plausible, since normal diploid human cells are typically refractory to transformation in vitro by simple, short-term overexpression of activated *ras* oncogenes [152,153]. However, the long-term overexpression of *ras* oncogenes has been associated with the destabilization of normal chromosomal structure by as yet unknown mechanisms [149–151]. A corollary to the assumption that *ras* oncogene-induced instability can eventually lead to the generation of complementing genetic lesions necessary for progression is that *ras* oncogenes should be able to complement existing genetic defects, with the end result again being cell transformation. Support for this prediction comes from studies showing that *ras* oncogenes can induce a full range of transformation-related traits in cells that either have a predetermined genetic abnormality (e.g., from patients with Bloom's syndrome) [154] or have undergone extensive chromosomal alterations as a consequence of prolonged passage in tissue culture [155].

We have tested the hypothesis that *ras* oncogenes can induce transformation of the human melanocyte by affecting, among other traits, genetic stability. We have previously observed that after introduction and short-term expression of the viral Ha- or Ki-*ras* oncogenes, cultured human diploid foreskin melanocytes acquire a number of characteristics seen in MM in vivo and in vitro [156,157]. These included altered morphology, anchorage independence, increased cell surface expression of G_{D3} ganglioside, induction of Class II major histocompatibility (MHC) antigens, and hyperploidy. However, other properties of melanoma, for example, loss of expression of the adenosine deaminase binding protein (ADAbp), alterations in differentiation programs, tumorigenic potential, and development of specific chromosomal abnormalities), were not observed. Further analysis, however, shows that the hypothesis outlined above is correct and that the introduction of a *ras* oncogene into a normal melanocyte results in genetic instability and in the eventual generation of a malignant melanoma cell that possesses all of the essential features observed in MM (AP Albino, unpublished). Thus, this in vitro system provides an excellent model for analyzing the sequence of phenotypic and genotypic events associated with complete neoplastic transformation of human melanocytes, and one that quite accurately mirrors the changes observed in melanoma in vivo. We will briefly review some of our observations with this transformation model.

Several specific changes can be detected when a *ras* oncogene is introduced into human melanocytes [156,158]. In the present discussion we will detail

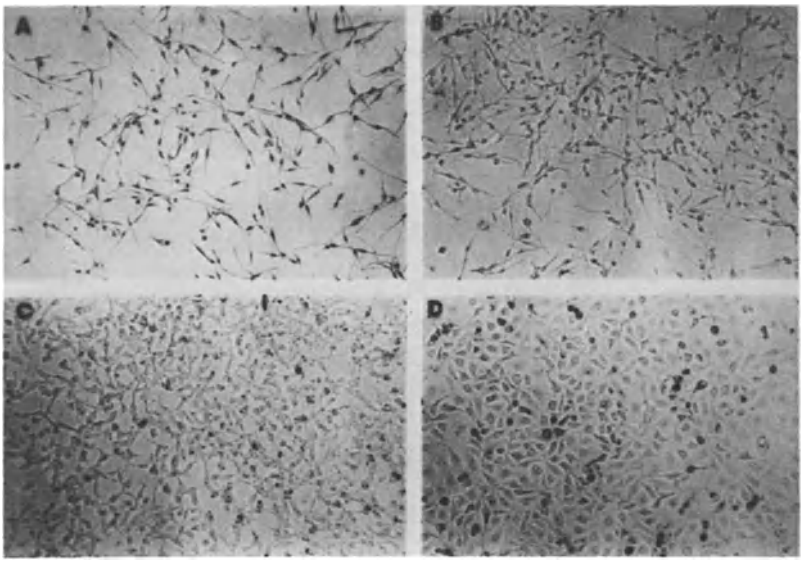

Figure 5. Photomicrograph of 10W melanocytes. A: uninfected; B: 10W*ras*/early; C: 10W*ras*/late; D: SK-MEL-28 melanoma.

the changes seen when foreskin melanocytes are transformed with the viral Ha-*ras* oncogene. Initially, these melanocytes undergo changes in morphology. Figure 5A shows the characteristic bipolar spindle-shaped morphology of normal melanocytes (culture 10W). Within 1 month after introduction of the v-Ha-*ras* oncogene, these melanocytes (termed *10Wras*/early) exhibited a polygonal morphology and tended to grow in clusters with increased cell-to-cell contact (Fig. 5B). 10W*ras*/early melanocytes maintained this morphological phenotype for approximately 6 months. Subsequently, foci of cells that had a distinctly different morphology spontaneously appeared in the culture (Fig. 5C). These morphologic variants, termed *10Wras*/late, displayed an epithelioid morphology, prominent cell-to-cell contact, and a propensity to grow to much higher saturation densities; this same constellation of traits is commonly observed in MM in culture (Fig. 5D).

Proliferation of melanocytes in vitro depends upon the addition of either the phorbol ester 12-o-tetradecanoyl phorbol-13-acetate (TPA) [159] or growth factors to the medium [160,161]. Of these, TPA is the most potent mitogen for melanocytes, and upon removal melanocytes rapidly become senescent. In contrast, cultured melanoma cells can grow vigorously in the absence of TPA, indicating that malignant transformation of melanocytes leads to autonomy from the growth-stimulatory effects of TPA. Uninfected 10W melanocytes and 10W*ras*/early cells maintained an obligatory growth requirement for TPA, senescing rapidly after removal of TPA. In contrast, 10W*ras*/late melanocytes did not senesce in the absence of TPA, but main-

tained their proliferative capacity. Moreover, uninfected 10W melanocytes maintained an anchorage-dependent phenotype and did not form colonies in semisolid agar (Table 4). 10W*ras*/early cells, however, formed growing colonies in agar (with a frequency of 0.25%), as did 10W*ras*/late melanocytes (with a frequency of 2% in the presence of TPA). In addition, injection of uninfected 10W melanocytes or 10W*ras*/early melanocytes into nu/nu mice did not form tumors. However, 10W*ras*/late melanocytes formed growing pigmented tumors in all mice injected, and thus, had acquired full malignant potential.

Using reverse-transcriptase-PCR methodology [162], we recently reported that the development of human melanoma is consistently associated with quantitative and qualitative alterations in the expression of several growth factors, which can be used as markers of transformation in this tumor type [163]. Uninfected 10W melanocytes, 10W*ras*/early, and 10W*ras*/late melanocytes were analyzed for the production of RNA transcripts for 10 different growth factors (results summarized in Table 4), and compared to the pattern observed in cultured melanomas [163]. Uninfected 10W melanocytes expressed transforming growth factors (TGF) TGFβ1, TGFβ3, keratinocyte growth factor (KGF), and platelet-derived growth factor A (PDGFA), which are characteristic of human melanocytes in culture [163]. In contrast, 10W*ras*/early and 10W*ras*/late melanocytes expressed RNA transcripts for all of these, in addition to TGFβ2, TGFα, and bFGF. This pattern of growth factor expression is identical to most cultured melanomas. One obvious conclusion from these data is that the induction of these additional growth factors in 10W*ras*/early cells is not sufficient for growth autonomy and independence from TPA.

Further changes in the expression of cell surface antigens occured during *ras*-induced melanocyte transformation [6,164,165]. Two of the most well-defined markers in this class are the 120 kDa ADAbp cell surface glycoprotein and Class II MHC antigens (e.g., HLA-DR). Melanocytes express ADAbp in vitro and in vivo, but ADAbp is not expressed by primary or metastatic melanoma cells [157]. Melanocytes are HLA-DR$^-$, but HLA-DR is constitutively expressed in a majority of primary and metastatic melanomas [166,167]. 10W melanocytes were HLA-DR$^-$ and expressed ADAbp. Consistent with our previous studies, expression of v-*ras* in 10W*ras*/early melanocytes led to induction of HLA-DR but not downregulation of ADAbp [156,157]. Moreover, there was a 5- to 10-fold increase in the surface expression of the disialoganglioside G_{D3} in these cells, which is an additional alteration consistently seen in melanoma cells [168]. In contrast, Table 4 shows that 10W*ras*/late cells not only maintained the induced expression of HLA-DR$^+$ and increased the expression of G_{D3}, but completely lost the expression of ADAbp. Extinction of ADAbp expression by 10W*ras*/late melanocytes correlated with a subset of traits, i.e., TPA independence, tumorigenicity, and (as will be discussed) the development of specific chromosomal markers, appearing late in the transformation process. 10W*ras*/late

Table 4. Phenotype of *ras*-transformed melanocytes

Trait	10W melanocytes	10W*ras*/early	10W*ras*/late	Melanomas
Morphology	Bipolar/spindle	Bipolar/spindle	Epithelioid	Epithelioid or spindle
Pigment	+	+	+	+ or −
TPA dependence	+	+	−	−
Anchorage dependence	+	−	−	−
Tumorigenicity	−	−	+	+
Karyotype	Diploid	Hyperdiploid	Aneuploid	Aneuploid
Marker chromosomes	None	i(6p), i(9q)	i(6p), i(9q), i(1q)	1,6,7,9
Antigen expression				
Class II MHC	−	+	+	+ or −
G_{D3} Ganglioside	+ (low)	+ (high)	+ (high)	+ (high)
EGFr	−	−	+	+ or −
ADAbp	+	+	−	−
CALLA	+	+	−	+ or −
C350 (gp180)	+	+	−	+ or −
TA99 (gp75)	+ (high)	+ (high)	+ (low)	+ or −
Growth factor expression				
TGFβ1	+	+	+	+
TGFβ2	−	+	+	+
TGFβ3	+	+	+	+
TGF	−	+	+	+
aFGF	−	−	−	+ or −
bFGF	−	+	+	+
FGF5	−	−	−	+ or −
KGF	+	+	+	+
PDGFA	−	+	+	+
PDGFB	−	−	−	+ or −

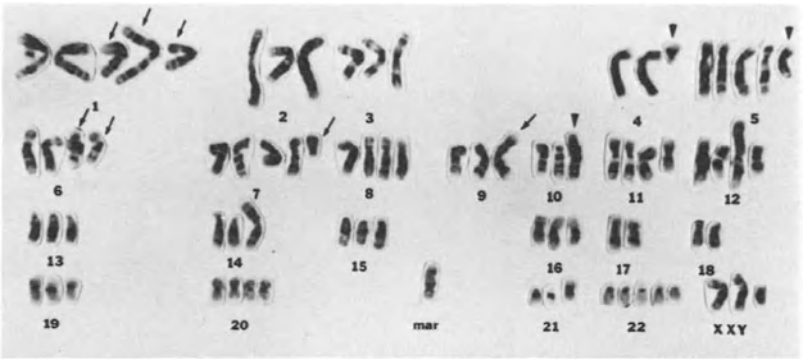

Figure 6. G-banded karyotype of 10W*ras*/late transformed melanocytes showing clonal abnormalities described in the text. The recurrent clonal abnormalities of chromosomes 1, 6, 7, and 9 seen in malignant melanoma are indicated by arrows. This cell also showed random loss of chromosome 9 and other nonclonal abnormalities of chromosomes (arrowheads). Chromosome preparations were analyzed using G-banding.

cells also manifested additional changes in the melanocyte differentiation program compared to parental 10W and 10W*ras*/early melanocytes. 10W*ras*/late melanocytes were epithelioid and lightly pigmented, distinct from the dendritic-spindle shape and deep pigmentation of 10W parental and 10W*ras*/early cells. Moreover, immunological analysis indicated a change in the differentiation program [148] of 10W*ras*/late cells from a mature melanocyte phenotype to a more immature melanocyte. 10W*ras*/late melanocytes showed expression of *EGFr*, but lost expression of late antigens gp180 (MabC350), common acute lymphoblastic leukemia antigen (CALLA) (MabAJ8), and the melanosomal membrane glycoprotein *TYRP* (MabTA99) [117,169,170].

Finally, uninfected 10W melanocytes exhibited a normal diploid karyotype in culture, with no detectable genetic abnormalities characteristic of cultured melanomas. Karyotype analysis of 10W*ras*/early cells, however, revealed a hyperdiploid karyotype with a chromosomal number ranging between 78 and 82. The chromosomal abnormalities included at least one copy each of i(6p) and i(9q), in addition to several structural rearrangements, trisomies, and tetrasomies of several different chromosomes (Table 4). A minor clone was also present with two copies of i(6p) and i(1q). In contrast, 10W*ras*/late melanocytes showed all cells had two copies of i(6p) and one copy each of i(9q) and i(1q) (see Table 4 and Fig. 6). The chromosomal complement of the major clone from 10W*ras*/late melanocytes was 78−82,XY,+X(3n±),+1,+5,−6,+7,−12,−14,−17,−18,+20,+22,del(10)(p13), +i(1q), +der(1)t(1;?)(p22;?), +2i(6p), +del(7)(cen), +der(8)t(8;?),(p11;?), +i(9q), +del(11)(q13q23), +der(12)t(12;?)(p13;?), +der(12)t(12;?)(q13;?), +der(14)t(14;15)(p11;q11),+i(21q),+mar.

Overall, the complex set of transformation-related changes acquired by v-Ha-*ras* infected melanocytes occurred in two phases. In the early phase, the

v-Ha-*ras* oncogene induced changes in morphology, anchorage-dependent growth, expression of class II MHC antigens, and the regulation of G_{D3} ganglioside. This was accompanied by a change in chromosomal ploidy and the appearance of i(6p) and i(9q). In the late phase, the most prominent alterations were loss of growth control, extinction of ADAbp expression, induction of tumorigenic potential, and selection of specific chromosomal alterations, such as del(1p). It has been documented that *ras* oncogenes can effect alterations in morphology, anchorage dependence [131], the control of class II MHC gene expression [156,171], and the deregulation of several disialogangliosides, including G_{D3} [156,172]. It is remarkable, however, that in vitro transformation of melanocytes by the Ha-*ras* oncogene mirrors a sequence of phenotypic and genotypic alterations observed during melanoma transformation and progression in vivo, especially since specific nonrandom chromosomal aberrations seen in vivo are usually not observed in in vitro model systems [173]. The earliest cytogenetic abnormalities observed in melanocytes expressing Ha-*ras* were the development of i(6p) and i(9q), followed by deletions of chromosome 1p in all the cells analyzed. As discussed above, numerous reports indicate that MM are characterized by severe aneuploidy with recurrent abnormalities of chromosomes 1, 6, 7, and 9 [7–10,53]. Thus, this model system accurately reflects the same chromosomal changes observed in vivo without the cells being subjected to in vivo phenomena such as immune surveillance.

In addition, the phenotypic and genotypic conversion of human melanocytes by a *ras* oncogene demonstrates that gene(s) on chromosomes 9 and 6, and possibly 1, may be critical to the development of malignant melanoma. This is the first experimental evidence to support the model, first suggested by cytogenetic and LOH studies [53], that an accumulation of several specific genetic alterations on these same chromosomes is necessary to induce the complete malignant transformation of the diploid melanocyte. In other studies in which the effects of oncogenes on melanocytes were analyzed, introduction of an adenovirus 12-SV40 hybrid construct into cultured melanocytes and nevus cells could also induce transformation and the acquisition of some of the characteristics of MM [174]. For example, these cells exhibited rapid proliferation, efficient growth in soft agar, loss of pigmentation associated characteristics, induction of Class II MHC genes, and increased G_{D3}/G_{D2} content. However, none of these cells were tumorigenic and none acquired specific chromosomal abnormalities commonly observed in melanoma in vivo, though they did show random structural and numerical aberrations.

In another study, the introduction of v-Ha-*ras* oncogene into a non-tumorigenic, but immortalized, line of murine melanocytes generated tumorigenic transfectants that no longer required TPA for continued growth [175]. The changes observed in these cells occurred several weeks after the introduction of the v-Ha-*ras* gene. These data suggest that the immortalized mouse cells had already completed the initial steps of transformation, and further support the interpretation that activated *ras* genes can complement

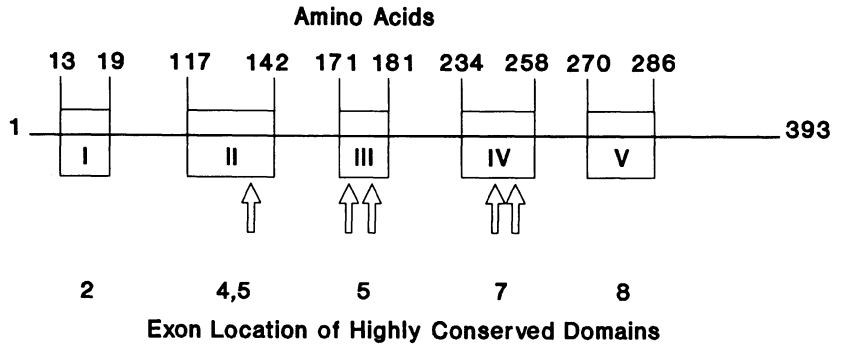

Figure 7. Structural organization of the human *p53* gene. Boxes I–V are the locations of highly conserved structural domains, which when mutated affect the tumor suppressor functions of *p53*. Arrows show the location of five different point mutations in the *p53* gene if five independent cell lines derived from metastatic melanomas.

pre-exisiting genetic defects in melanocytes, with the end result being malignant transformation.

It has also been shown that the introduction of the polyoma middle T gene into murine melanocytes can induce independence from TPA and tumorigenicity [176]. In this case, the precise mechanisms, as well as the cellular and biochemical pathways affected by activated *ras* genes in these cells, are beginning to yield to analysis. For example, middle T antigen has been shown to form a complex with the c-*yes* oncoprotein [177]. We have preliminary evidence that the regulation of the c-*yes* gene (see below) is perturbed in a majority of metastatic melanomas. Thus, these data draw attention to c-*yes* and the downstream pathways interdicted by the c-*yes* oncoprotein. Finally, in a powerful new model for studying the interactions of oncogenes and MM formation, it has recently been shown that introduction of the SV40 early region gene under the control of the tyrosinase promoter caused the development of metastatic melanomas in transgenic mice [178]. Further exploitation of this system should prove exciting.

6.1. Summary

What is the in vivo impact of *ras* oncogenes? Involvement of *ras* oncogenes in the initiation of a majority of melanomas is doubtful. However, it appears likely that *ras* oncogenes can have a profound impact on differentiation, and it remains to be determined what clinical significance this may have. The dramatic impact of *ras* oncogenes on the melanocyte in vitro could prove useful for the detection of novel genes directly involved in the pathogenesis of melanoma. Moreover, the use of models of transformation provides an opportunity to study and possibly to detect early genetic and biochemical

abnormalities that may be lost or obscured in the study of primary and malignant melanomas from patients.

7. The *p53* gene and melanomas

The *p53* tumor suppressor gene, located on chromosome 17p, is the most frequently deranged gene in a variety of human cancers [132]. The most commonly detected defects in this gene are point mutations in highly conserved structural domains that affect the tumor suppressor functions of the *p53* protein. We have evaluated the prevalence of mutations in the *p53* gene in cultured and noncultured specimens of metastatic melanoma by direct sequence analysis and by staining of melanoma specimens with anti-*p53* monoclonal antibodies in avidin-biotin-peroxidase immunohistochemical reactions.

Our analysis of *p53* point mutations has relied on three different techniques. First, we have used a variation of the method, termed single-strand conformation polymorphism (SSCP) analysis. As shown by Orita et al. [179], conformation polymorphisms of single-stranded DNA (ssDNA) can be used to detect point mutations (DNA-SSCP). A recently developed related method allows the use of cDNAS reverse-transcribed from mRNA, followed by PCR amplification and subsequent transcription back into RNA (termed *RNA-PCR-SSCP*) [180]. The transcribed RNA is analyzed for conformation polymorphisms in polyacrylamide gels. One advantage of this technique is that RNA has more conformational polymorphisms than ssDNA and the likelihood that a single nucleotide alteration results in another conformation is higher.

We prepared cDNAs from RNA pools extracted from 35 cultured metastatic melanomas, amplified the cDNAs for all of the coding regions of *p53*, then transcribed these *p53*-specific amplified fragments back into RNA, and analyzed them on polyacrylamide gels. This method detected mutations in 20% of melanoma cell lines in several of the regions conserved in the *p53* gene (Fig. 7). Direct sequence analysis confirmed the presence and specificity of each *p53* mutation detected by RNA-PCR-SSCP. The majority of mutations were GC → TA transversions, rather than CC → TT transitions, which are known to be caused by UVR. Thus, unlike squamous cell carcinoma, in which the majority of tumors contain this type of UVR-induced mutation [181], the mutations in the *p53* gene in MM do not appear to be UVR specific.

We have also used direct PCR amplification and sequencing of genomic DNA from paraffin-embedded biopsies to screen melanomas for *p53* mutations [182]. The advantage of this technique is the ability to perform retrospective studies on archival material, and on specimens, such as atypical nevi and primary melanomas, for which tissue availability is limited. Our preliminary results to date show similar results to that seen with RNA-PCR-

SSCP in that approximately 15% of melanomas harbor mutations in the *p53* genes (AP Albino, unpublished). In terms of protein expression, the unmutated *p53* protein is restricted to the cell nucleus [183,184] and is usually undetectable in normal cells. In contrast, mutated *p53* can appear in the cytoplasm as well as in the nucleus, and is detectable by immunohistochemical methods in both these cellular compartments. In our study of melanoma cell lines using antibody 1801 (this antibody recognizes a denaturation-resistant epitope between amino acid residues 32 and 79 of human wild-type and mutant *p53*) [185], cell lines with *p53* mutations showed strong immunoreactivity in the majority of cells, with granular staining being most intense in the nucleus and less intense in the cytoplasm.

The finding that the *p53* gene is mutated in human melanomas raises the possibility that this gene may be involved in the etiology of a subset of MM. Alternatively, it is possible that inactivation of *p53* by mutation may play no fundamental role in the pathology of MM, but is due simply to the genetic instability in MM cells. When *p53* mutations are detected, they are not localized to any particular 'hot-spot' region, indicating that the environmental or metabolic insult inducing *p53* mutations does not affect any one specific portion of the *p53* coding sequence. In contrast to our findings, a recent paper [186] has reported that 85% of specimens from a range of primary and metastatic melanomas have increased prevalence of mutant *p53* protein as detected by immunohistochemistry. At present it is difficult to resolve these findings with our own. In the immunohistochemical study, no attempt was made to confirm the presence of *p53* point mutations by direct DNA sequencing. We have used three different methods to detect alterations both in the expression and in the DNA sequence of all of the coding domains of *p53* in MM. Our results are consistent in finding a much lower frequency of mutant *p53* protein and mutated *p53* gene. Clearly, further analysis is needed to resolve this issue.

8. Expression of growth factor related genes in melanomas

In addition to activation of oncogenes and inactivation of tumor suppressor genes, neoplastic transformation of human cells is also consistently associated with quantitative or qualitative alterations in the expression of growth factors [45,187]. These factors are thought to confer a growth advantage upon the malignant cell by acting as autocrine and paracrine mediators of cellular proliferation. In an attempt to detect perturbations in growth factor regulation between normal and malignant melanocytes, we have analyzed a series of cell lines established from human metastatic melanomas and normal foreskin melanocytes for the production of RNA transcripts specific to a spectrum of growth factors, including transforming growth factors (TGFα, TGFβ1, TFGβ2, TGFβ3), fibroblast growth factors (acidic-FGF, basic-FGF, FGF-5, and HST), keratinocyte growth factors (KGF), and platelet-derived growth

factors (PDGFA and PDGFB). The results indicated clear distinctions in the patterns of growth factor expression in these cell types and have focused attention on several growth factors that may have etiologic significance and that can be used as markers of transformation. Virtually all melanoma cell lines expressed RNA transcripts for TGFβ1 (18/19), TGFβ2 (18/19), TGFβ3 (19/19), TGFα (18/19), bFGF (19/19), KGF (18/19), and PDGFA (15/19). A subset of cultured melanomas also expressed aFGF (3/19), FGF-5 (5/19), and PDGFB (2/19). No melanoma culture (0/19) expressed HST-specific RNA transcripts. In contrast to these melanoma cell lines, cultured melanocytes had a different pattern of RNA expression. Melanocytes expressed TGFβ1 (14/14), TGFβ3 (14/14), and KGF (11/13), but there was no detectable expression of TGFβ2 (0/14), aFGF (0/13), bFGF (0/14), FGF-5 (0/13), PDGFB (0/13), or HST (0/13). A subset of melanocyte cell lines did express PDGFA transcripts (5/14), as well as low levels of TGFα transcripts (3/13). These results have been confirmed by other studies of melanomas and melanocytes [188,189].

TGFα, a 50 amino acid polypeptide that shares high structural homology in the receptor binding domain with EGF, can bind and activate the EGFr [190], thereby stimulating proliferation by an autocrine-mediated pathway [191]. TGFα is expressed by a range of normal and malignant human tissues [192–195]. Overexpression of TGFα can induce the malignant transformation of immortalized cells in vitro [196]. Our results showed a qualitative difference in the expression of TGFα between melanocytes and melanoma cells. Abnormal expression of TGFα has been documented in melanomas [192,193,197], and TGFα can stimulate the growth of both melanomas [198,199] and melanocytes [198] in vitro. Low-level to no transcription of TGFα was observed in several melanocyte cultures, whereas all cultures of metastatic melanomas produced large amounts (5- to 10-fold more) of TGFα RNA transcripts. The fact that some melanocyte cultures expressed TGFα may indicate that, as in keratinocytes [194], this factor may also have a normal physiological role in autocrine/paracrine stimulation. In addition, the presence of a rare restriction fragment length polymorphism for the TGFα gene in melanoma patients, but not in normal individuals, may be a risk factor for this disease [199].

The TGFβ family, which consists of at least three distinct members (TGFβ1, TGFβ2, and TGFβ3) [200–202], can both inhibit and stimulate cell proliferation and influence differentiation [203,204]. TGFβ1 is ubiquitously expressed in human tissues, while expression of TFGβ2 and TGFβ3 varies with cell type [192,194,200,205]. We showed that cultured normal melanocytes and malignant melanomas express TGFβ1 and TGFβ3, but that only melanoma cells synthesize TGFβ2 RNA transcripts. Since both normal and malignant melanocytes express TGFβ1 and TGFβ3, it is possible that these genes are involved more in the normal growth and differentiation of melanocytes than in melanoma development. In contrast, the transcription of TGFβ2 in all melanoma cell lines studied indicates that this gene is regulated

independently of TGFβ1 and TGFβ3, and may play a critical role during the development of the melanoma cell. TGFβ2 does have immunosuppressive effects [206], and it has been speculated that TGFβ2-producing glioblastomas may escape immune surveillance by inducing immunosuppression in the host [207]. TGFβ1, which shares a wide range of biological effects with TGFβ2 [208–211], has been shown to affect the regulation of expression of Class II histocompatibility antigens in melanomas, and it is speculated that this regulation also has an adverse effect on host immune response [212]. Thus, by comparison to TGFβ1, TGFβ2 may similarly impact on the growth and maintenance of melanoma cells. While it remains to be determined if all three TGFβ mRNAs are actually translated into functional protein, the qualitative alteration in TGFβ2 transcription upon malignant transformation of the melanocyte may allow its use as a new marker of transformation in MM. Whether the induction of TGFβ2 expression is associated with initial transforming events or with secondary events (e.g., those that affect growth, maintenance, or suppression of host immunity) is unknown.

The fibroblast growth factor (FGF) family is a diverse group of polypeptide growth factors characterized by structural and amino acid sequence homology, heparin binding characteristics, and the ability to promote angiogenesis as well as to provide mitogenic stimuli for a wide range of mesoderm and ectoderm-derived cell types [213,214]. The FGF family includes at least seven distinct members: bFGF, aFGF, FGF-5, HST/K-FGF, FGF-6, Int-2, and KGF. We have determined the transcription patterns of five of these polypeptides, bFGF, aFGF, FGF-5, HST, and KGF. Virtually all melanomas expressed transcripts for bFGF and KGF, while a subset expressed aFGF and FGF-5, and none expressed HST. bFGF, located on chromosome 4q25, is expressed in a wide range of cell types and tissues. Under certain conditions bFGF can act as an autocrine mediator of cell proliferation and as a transforming oncogene when overexpressed intracellularly or when fused with a signal peptide and secreted [215]. These biological properties support the idea that bFGF can mediate neoplastic growth and the development of metastasis by a variety of mechanisms.

The induction of bFGF transcription in all melanomas examined suggests that it plays an important role in proliferation or in development of the malignant phenotype. This interpretation is strengthened by the observations that normal melanocytes require exogenous bFGF to proliferate in culture [161,216] and that the growth of melanoma cells in culture can be inhibited by suppressing endogenous bFGF synthesis with antisense oligonucleotides [217]. However, while the spontaneous production of bFGF by transformed melanocytes may allow unrestrained proliferation, it is unlikely that bFGF production is an initiating event, since transfection of bFGF cDNA into murine melanocytes abrogates proliferation dependence on exogenous bFGF but does not induce tumorigenicity [189,218].

Transcripts of FGF-5 [219], a potent mitogen for fibroblasts and endothelial cells, could not be detected in any melanocyte culture, whereas FGF-5 could

be detected in about 25% of melanomas. The expression of FGF-5 in a subset of MM indicates that the transcription of this gene may be a useful marker of transformation. Similarly, a subset (15%) of melanoma cultures synthesized aFGF transcripts. aFGF can transform cells when overexpressed [220], presumably by acting as an autocrine mediator of cell proliferation [213]. It is interesting to note that there were qualitative differences in the expression of aFGF among four cell lines established from separate metastatic deposits of an individual patient. Of these four lines, two expressed aFGF RNA and two had no detectable expression. The two lines not expressing aFGF were established from metastases biopsied 2 years before those used to establish the two lines that did express aFGF [78,221]. Thus one conjecture is that aFGF correlates with very late stages in tumor progression.

In conclusion, one of the most common defects in the development of neoplasms is the transcriptional activation of a set of proto-oncogenes encoding growth factors and their receptors [2,45,222]. It is hypothesized that these genes have both direct and indirect roles in regulating the growth of a particular tumor. The development of the MM cell is associated with the induction of TGFβ2, TGFα, and bFGF. It is interesting to note, however, that none of these genes is located on chromosomes targeted by cytogenetic and LOH studies as the location of genes critical to the development of MM (see Fig. 3). Obviously, the continued analysis of these genes and other growth factor related genes in both premalignant lesions and malignant melanomas is necessary for ultimately determining their relevance in the development and progression of the melanoma cell.

9. Involvement of other oncogenes in MM

Defining the specific genetic changes in proto-oncogenes for each tumor type is of fundamental importance [45]. A number of proto-oncogenes map to frequently targeted chromosomal regions in MM: e.g., chromosome 1p (*fgr*, *lck*, *L-myc*, *N-ras*, *jun*, *rap1a*, *tcl-5*, *clk-1*), 6q (*ros-1*, *myb*, *mas-1*, *fyn*); chromosome 7 (*erbB1*, *met*); and chromosome 9p (*N-rasL1*), as well as to infrequently targeted regions of these chromosomes, e.g., *ski* and *trk* on 1q, *pim* on 6p [19]. However, there is little direct evidence to suggest a critical influence of any of these genes in the pathogenesis of either familial or sporadic MM. With the exception of the N-*ras* gene (see above), the most consistent abnormality associated with some of these genes, as well as others not located on these chromosomes, is an alteration in their expression. Moreover, the majority of studies published to date have focused on cell lines derived from metastatic melanomas, and it remains to be determined if defects in the expression of these genes are associated with other stages in the development of MM.

As discussed above, the most commonly mutated oncogenes are members of the *ras* gene family. However, these are mutated in only a subset (i.e.,

12–24%) of primary and metastatic melanomas, and data to date suggest that these are late events in the progression of MM. The *p53* tumor suppressor gene is also found to be mutated in a subset of invasive primary and metastatic melanomas. There is, as yet, no published data detailing the occurrence of *p53* mutations in atypical nevi or primary melanomas in the radial growth phase. Consequently, it is too early to comment on whether *p53* alterations may be directly involved in the initiation of a subset of primary melanomas. Several studies have analyzed a wide range of oncogenes, growth factors, and their cognate receptors, and have also failed to find a strong association for any gene amplification, rearrangement, mutation, or other structural defect in a large series of cultured and noncultured primary and metastatic melanomas [103,189]. A rare example of a consistent chromosomal aberration correlating with a detected defect in a specific growth-related gene is seen in the elevated expression of the EGFr [64], apparently caused by amplification of chromosome 7. However, overexpression of EGFr appears to impact more on invasion and metastasis than initiation [223].

The most recurrent and consistent type of gene perturbation in MM appears to be altered expression. The present supposition is that these genes are altered, not in their coding domains, but in the regions that control transcription and translation. Evidence detailing the specific defects responsible for the observed deregulation in these genes (e.g., mutation), however, is still lacking. As the subject of growth factors will be reviewed elsewhere in the monograph, we will only briefly highlight some studies here. We and others have shown that there is deregulated expression of bFGF, TGFβ2, TGFα, PDGFA, and melanoma growth-stimulating activity (MGSA) in most metastatic melanomas, and of aFGF, FGF-5, PDGFB, and interleukin (IL) IL-1α and IL-1β in a subset of melanomas (for review see ref. 189). There are also some data to suggest that there may be deregulation of various members of the *c-fos* and *c-jun* families of transcriptional activators [224] (Ap Albino, unpublished).

In addition, as with other malignancies [225,226], emerging data suggest a connection between defects in protein tyrosine kinases and altered signal transduction in the pathogenesis of MM [227,228]. For example, the proto-oncogene c-*kit* is a receptor-type protein tyrosine kinase located on chromosome 4q. In combination with its ligand, c-*kit* appears to play an essential role in the normal growth and differentiation of melanocytes [229,230]. While the c-*kit* protein is abundant in normal melanocytes, it is markedly reduced or absent from a majority of melanoma cell lines and from melanocytes transformed in vitro [227]. Thus, it is possible that perturbations in signal transduction caused by the loss of c-*kit* may have a role in the pathology of MM. It is also possible that the maintenance of the human epidermal melanocyte in a differentiated, nonproliferative state is dependent on the production of c-*kit* and its ligand. Loss of either of these polypeptides may complement other defects, leading to disruptions in differentiation programs and the homeostatic mechanisms controlling melanocytes.

Another transmembrane tyrosine kinase protooncogene, c-*ret* [231], which is related to c-*kit*, can cause aberrant melanogenesis and melanocytic tumor development in transgenic mice that carry a metallothionein/*ret* fusion gene [232]. However, expression of the *ret* proto-oncogene is limited in human neoplasias [233]. Similar to c-*kit*, protein expression of another member of the tyrosine kinase growth-factor gene family, c-*met*, is also downregulated or extinguished in MM [189]. Therefore, like c-*kit*, lack of expression of c-*met* in MM could be a meaningful defect in the development of MM. Though c-*met* was originally described as a dominant oncogene due to its transforming potential in the NIH3T3 cell [234,235], its true physiologic role in other cell types remains to be elucidated.

We have preliminary evidence that the c-*yes*-1 gene, another protein tyrosine kinase, is also perturbed in a majority of MM cell lines. The c-*yes* protooncogene encodes a protein tyrosine kinase (PTK), $p62^{c\text{-}yes}$, that is structurally and functionally related to the *src* family of non-receptor-type PTKs and is located on chromosome 18q [236,237]. Activation of the transforming potential of the *c-yes* gene correlates with an increase in its PTK activity [238]. We compared the levels of $p62^{c\text{-}yes}$ protein and kinase activity by immune blot and immune complex kinase assays in 20 human melanoma and seven melanocyte cell lines (Ap Albino, unpublished). Results show that the relative levels of cytoplasmic $p62^{c\text{-}yes}$ protein is altered in melanomas in comparison to melanocytes. The increase $p62^{c\text{-}yes}$ protein correlates with a large increase in $p62^{c\text{-}yes}$-specific kinase activity (5- to 10-fold) in 18 of 20 melanoma lines. Single-strand conformational polymorphism analysis of all structural and functional domains detected a possible mutation in a proportion of these melanomas at the carboxyterminus of the c-*yes* protein, an area that contains a regulatory tyrosine. In addition, a second potential transformation-related alteration seen in these melanoma lines were defects in the normal cellular compartmentalization of the $p62^{c\text{-}yes}$ protein. These data suggest that derangement of the c-*yes* PTK may have a role in the transformation process in MM.

Thus, at present, the precise biochemical and biological role of the above-mentioned genes in the pathogenesis and progression of MM remains to be elucidated. However, the studies to date suggest a broad array of disturbances in MM involving transcriptional regulators, growth factors, growth factor receptors, dominant oncogenes, tumor suppressor genes, genes that control phosphorylation of proteins, and genes that control entry into the cell cycle. One can only speculate as to the complexity and myriad interactions that may be disrupted during the transformation and progression of the human melanocyte. The eventual identification of a subset of genes that are perturbed at the genetic and biochemical levels will obviously allow a clearer picture of the specific roles these genes may play in the pathogenesis of MM.

10. Impact of UVR

UVR, specifically ultraviolet radiation B (UVB), which is composed of wavelengths between 280 and 315 nm, has long been considered a risk factor in the development of MM [239,240]. However, while the dramatic increase of sporadic melanoma in fair-skinned people is believed to be directly linked to UVR exposure [241], the precise molecular impact of UVR in the pathogenesis of MM remains obscure. This is because the suspected risk of UVR carcinogenesis in humans is based mainly on epidemiological studies [12]. In addition, data from experimental model systems of UVR induction of squamous cell carcinoma in mice and melanoma in the South American opossum are not directly transferrable to humans, due to basic differences in the biology of these species and to the relatively high doses of UVR needed to induce transformation in these animal models. Consequently, defining the precise interactions of normal human skin cells and UVR during the process of transformation has been quite complex. The indisputable fact is that UVR can damage DNA. The concomitant biological and biochemical results of this damage are less clear. Two broad areas of inquiry have focused on determining the direct genetic alterations (e.g., mutations and other perturbations) of UVR and the indirect effects of UVR on the host (e.g., the immune system, the local microenvironment, etc.), which may induce a milieu more conducive to the growth of premalignant or malignant cells.

UVR has several known effects on DNA, the most common being the formation of chemical bonds between two adjacent pyrimidines, particularly thymidine [242]. Another more menacing type of UVR-induced dimerization occurs between adjacent thymidines and cytosines, as opposed to thymidine dimers. This type of dimer can be mutagenic. Dimerization induced by UVR of 260 nm causes the position of these bases in the DNA helix to be so displaced that they can no longer properly base-pair with opposing bases. Skin cells are believed to generate a large number of UVR-induced pyrimidine dimers per day [242] that are efficiently removed by repair enzymes [243]. Failure to repair this damage can have dire consequences, as is clearly shown in studies of individuals afflicted with the rare inherited disease xeroderma pigmentosum [244,245]. These individuals are homozygous for a recessive mutation that inactivates the gene involved in removal of UVR-induced dimers [246] and, as a result, have a dramatically increased (on the order of 1000-fold) incidence of skin neoplasms, including basal cell carcinomas and MM [247,248]. Clearly, there is a direct link between simple repair of DNA dimers and prevention of most malignant skin cancers [249]. Therefore, this naturally occurring genetic disorder allows a critical scrutiny of the interplay of specific DNA abnormalities and disease, and has resulted in a number of observations detailing the particular types of faulty repair mechanisms in XP cells, and by analogy to normal epidermal cells.

A recent study has attempted to further define the types of observed

defects in the repair mechanisms of XP and normal cells, and has re-examined the relationship between the rate of excision repair and a biological end point, namely, cell survival [245]. The results of this study showed that repair in XP cells from different complementation groups involves either reduced availability of an essential repair enzyme or an abnormally reduced amount of chromatin substrate to repair. In the latter case, it would appear that some factor(s) that controls the accessibility of active gene regions for preferential repair is perturbed.

The presumption of this and other studies [247,248] is that incomplete repair of UVR damage in the DNA from MM patients is qualitatively, but probably not quantitatively, similar to the reduced accessibility case, since the repair enzyme in MM patients is not deficient. Therefore, one part of the puzzle may be that UVR induces DNA damage in epidermal melanocytes that is repaired faultily, repaired too slowly, or not repaired at all. The consequences of the resulting spectrum of genetic defects must have a myriad of effects, including, presumably, the generation of initiated or transformed melanocytes.

A second risk factor for MM is the type of melanin an individual possesses. Eumelanin, a relatively stable molecule, predominates in people with darker skin [250], while pheomelanin accounts for a larger percentage of melanin molecules in light-skinned people. Pheomelanin is highly photolabile and releases an oxygen free radical following UVR exposure [251]. Pheomelanin-related UVR byproducts are mutagenic in the Ames test [252]. These observations have prompted a proposal that melanoma may arise through 'autocarcinogenesis' by which the production of free radicals or oxidants produced during melanogenesis, possibly augmented or deranged by UVR, could initiate the primary events necessary for carcinogenesis in the melanocyte [253].

Normal epidermal melanocytes divide infrequently in the skin. However, UVR exposure results in a complex series of normal events that leads to a transient proliferation of epidermal melanocytes in vivo [254,255] and in vitro [256]. The mechanism by which UVR stimulates melanocyte proliferation is unknown but is thought to act through second-messenger signal transduction systems, such as cyclic-AMP and protein kinase C, since chemical agents that stimulate these pathways also induce the proliferation of epidermal melanocytes [216,218,257]. Several reports have shown that UVR in vitro causes a decrease in the binding of EGF to its receptor in murine fibroblasts, as well as stimulating arachidonic acid deacylation and arachidonate metabolite production in human epidermal cells [258–261]. TPA, the most potent growth promoter for melanocytes in vitro, has similar effects on EGF binding and arachidonic acid metabolism. Consequently, UVR may promote the growth of melanocytes by similar mechanisms. Upon the malignant transformation of human melanocytes, the dependence on TPA ceases [159] and cultured melanoma cells can grow vigorously in the absence of TPA, indicating that malignant transformation of melanocytes leads to autonomy from the

growth-stimulatory effects of TPA. Whether UVR and TPA are impacting on similar biochemical pathways remains to be determined.

In addition to PKC activation, UVR stimulation of cell growth must, presumably, involve other types of gene products, e.g., mitogenic growth factors, macromolecules involved in propelling the cell through the cell cycle, cell attachment proteins, etc. As these products are components of the same systems that go awry in neoplasias, a study of how a normal cell manages its proliferation and intercellular relationships is of extreme importance. Accordingly, it has been shown that UVR can induce the production of transforming growth factor-α (TGF-α) in epidermal melanocytes [198], and it has been hypothesized that this induction is linked to the promotion of initiated preneoplastic or neoplastic cells [198]. The activation of this gene, in concert with other nuclear proteins (e.g., *p53*, *myc*, *fos*, etc.), may result in an autocrine-type positive growth regulatory process, propelling the melanocyte into an actively proliferating mode, accompanied by routine DNA repair maintenance. These data also suggest that TGF-α may participate as an autocrine mediator of UVR-induced tumor promotion; and not surprisingly, TGF-α production is both inducible and constitutively produced in melanomas [163,192,193,197], and overexpression of this molecule can both transform cells and stimulate proliferation by an autocrine-mediated pathway.

UVR can be both an initiator and promoter of MM, and is thus labeled a 'complete carcinogen' [262]. UVR can induce mutations in DNA and act as an independent initiator of cell division [255,256]. If the cell cannot effect adequate repairs to the DNA, the ability of UVR to stimulate cell growth could allow the propagation of potentially mutagenic DNA damage while permitting additional damage to collect. But what types of damage are critical and what are the biological effects of this damage? Clearly, the vast majority of fair-skinned people do not get MM, even when spending a lifetime in regions of high UVR, such as Australia [263]. While it is reasonable to assume that genotype and other types of epigenetic phenomena may play a large role in protecting various individuals from neoplastic development, it is also likely that, like most cancers, there is a cell type and/or lineage-type of specific damage that must accrue before the development of a neoplasm at any particular site occurs. Moreover, this damage must be devastating to a particular cell. It is easy to speculate that many types of DNA damage will not initiate a neoplasm. One can envision the presence of epidermal melanocytes harboring a critical, but biologically inert, genetic lesion due to prior UVR damage. If this initiated melanocyte subsequently acquired a potentially complementing lesion, the transformation process could proceed.

Alternatively, the initiated melanocyte could be promoted by potential regulatory factors, such as TGF-β, that may have an adverse effect on surrounding (and constraining) normal cells, thereby allowing clonal outgrowth of preneoplastic cells [264]. In this regard, UVR has been shown to

have a stimulatory effect on the outgrowth of melanoma cells in mouse model systems [265]. This stimulation is primarily caused by effecting alterations in the host response and may include UVR damage to cutaneous immune cells, resulting in transient suppression of immunological defense mechanisms and/or local release of melanocyte-stimulating growth factors [266–269]. Suppression of T-lymphocyte activity by UVR appears to prevent immune rejection of skin tumors in mice [270]. Other studies showing that immunosuppressed transplant patients have an increased rate of squamous and basal cell carcinomas clearly suggest that interference with normal host immune systems is involved in the development of skin neoplasias [271]. Thus, the impact of UVR on immune surveillance of premalignant or malignant cells could be of major significance in the development of MM.

As discussed above, UVR could induce point mutations in specific dominant-acting oncogenes that can stimulate cell proliferation and/or destabilize normal mechanisms, leading to genetic instability and setting the stage for the slow, but inexorable, accumulation of genetically advantageous mutations. A subset of cultured and noncultured melanocytic tumors contain point mutations that activate the transforming potential of *ras*-encoded p21 proteins. Greater than 90% of these mutations occur at the 61st codon in the second exon of the N-*ras* gene. One possibile explanation for this specificity may be that UVR [241] preferentially targets the N-*ras* gene in vivo. This supposition has been supported by a recent paper that showed N-*ras* is the only *ras* gene mutated in a subset of primary melanomas originating in sun-exposed sites, suggesting a direct etiologic link between UVR and N-*ras* point mutations with transforming potential [139]. In addition, it has been shown that UVR mutates the N-*ras* gene predominantly at the 61st codon in vitro [272]. In mouse model systems, epidermal papillomas and carcinomas were induced on the skin of Sencar mice after exposure to a single dose of UVR, and amplification and overexpression of the N-*ras* related c-Ha-*ras* gene was implicated as being necessary but insufficient for completion of the progression towards full malignancy [273]. Similarly, our model system using *ras* oncogenes to transform human melanocytes shows that *ras* genes may act by inducing genetic instability, which, when complemented by the appropriate secondary genetic aberrations, can complete the transformation process, with the melanocytes acquiring all the phenotypic and genotypic characteristics usually seen in melanomas in vivo.

Ambiguity about the importance of *ras* gene involvement also becomes apparent upon analyzing XP cells. Since these cells have a dramatically diminished repair mechanism that, among other defects, is deficient in repairing UVR-induced DNA damage, one could expect XP tumors to have a much higher frequency of *ras* gene mutations than sporadic melanomas. *Ras*-activating point mutations, presumably the result of UV-induced pyrimidine dimer formation, are found in the 61st codon of the second coding exon of the N-*ras* gene in only 25% of XP tumors [274], which is similar to the frequency of N-*ras* mutations seen in sporadic cases of cutaneous primary

melanomas [139] and squamous cell carcinomas [275] arising in sun-exposed sites. While these results cannot, as yet, be unified into a single hypothesis of *ras* oncogene involvement, one possible scenario is as follows. Mutated and activated *ras* genes alone cannot cause the neoplastic transformation of precursor cells in vivo [131,276,277]. However, the activation of *ras* oncogenes can induce a complex variety of effects, including alterations in differentiation programs and the destabilization of normal chromosomal structure [278]. If through this destabilization the cell acquires other complementing and advantageous genetic abnormalities, then this could set the stage for the complete transformation of the cell and the development of a skin neoplasm [279]. However, since the frequency of mutated *ras* genes in both XP cells and MM is relatively low, there must be alternative pathways to transformation. Consequently, in the majority of skin neoplasms there must be genes other than *ras* whose derangement is critical. It is an assumption that these unknown genes would also be adversely affected by UVR. The determination of the precise complement of deranged genes (e.g., *ras*) and other host genes that satisfy the minimal requirements for transformation remains the fundamental task.

Equally important to studying damaged genes, however, will be studies detailing the complex response of the host to environmental insults. One of the more important observations concerning the interplay of environment and *ras* oncogenes shows that a normal cellular environment can suppress tumor formation induced by *ras* oncogenes [280,281]. In a mouse skin model system in which activated *ras* oncogenes were introduced into keratinocytes and regrafted back into syngeneic animals, the surrounding normal tissue fibroblasts were found to supply unknown factors that constrained the development of carcinomas [282,283]. The seminal and testable idea in these studies is that the transforming effects of a single oncogene can be neutralized (but presumably not abrogated entirely) if the damage occurs in a single cell that is surrounded by a normal tissue milieu.

This suppression may be due to growth inhibitors produced by the normal cells and are, therefore, the products of tumor suppressor genes. However, if there is activation of multiple oncogenes in a single cell, and/or a breakdown in normal intercellular interactions and/or destruction of normal tissue architecture, then this rogue cell would not be constrained by surrounding normal cells and could be free to progress to a preneoplastic or neoplastic lesion. Cells within this lesion could spontaneously undergo further progression and eventually generate invasive and metastatic variants [284]. It is fascinating to speculate that high-dose UVR alone could both mutate individual genes and cause a local disruption of normal tissue architecture. Various types of minimal damage to surrounding tissues could be complemented by the induction of cell surface molecules, which may disrupt the normal cellular interactions between skin cells such as melanocytes, keratinocytes, Langerhans' cells, etc. Subsequent UVR or chemical damage could provide the secondary steps in the transformation process or further disrupt the local

environment, so that now the cell containing a mutated oncogene is released from constraints. It is likely that future studies with in vitro model systems will give insight into the nature of UVR-induced genetic abnormalities in skin neoplasms, the relationship of *ras* oncogenes to transformation and differentiation, and the involvement of the host in tumor formation.

Similar to *ras* genes, mutational activation of the *p53* tumor suppressor gene may also be involved in the pathogenesis of a subset of melanomas. It remains to be determined if *p53* mutations are directly related to UVR exposure, but alteration of *p53* can affect the transition of cells into active proliferation [285]. UVR has been implicated in *p53* mutations and in the overexpression of *p53* protein in sun-damaged skin, actinic keratoses, basal cell carcinomas (BCC), and squamous cell carcinomas [181,286]. BCC is the most common type of human cancer and the most common neoplasm arising on the head, neck, and other chronically UVR-exposed regions of fair-skinned people in the fourth decade of life and later. Chronic UVR exposure is the most importance etiologic factor identified in most cases of BCC [287], but as in MM, the subsequent molecular-genetic steps in the progression of BCC are poorly understood. In BCC, in contrast to MM, overexpression of *p53* protein was noted in 83% of noncultured specimens [286].

Keratinocytes of chronically sun-exposed epidermis adjacent to BCCs, as well as actinic keratoses, also focally expressed *p53* protein in the majority of cases, while those of sun-protected skin did not. It is thought that chronic UVR exposure is necessary for the development of BCC, whereas short, punctate bursts of UVR during one's lifetime is a risk factor for MM. Mutations in the *p53* gene in sun-damaged keratinocytes may occur early in the pathogenetic sequence, eventuating in the formation of BCC. While it remains to be determined precisely when during MM tumor progression that *p53* mutations occur, the fact that *p53* mutations are detected in only a subset of MM (<25%) may mean that this gene plays a different role in MM than it does in BCC. What is clear is that the *p53* mutations identified to date in MM (see Fig. 7) are not localized to any particular 'hot-spot' region. This fact indicates that the environmental (UVR?) or metabolic insult inducing *p53* mutations in MM does not affect any one specific portion of the *p53* coding sequence, implying a more general field effect of the putative mutagenic agent.

The ability of UVR to both mutate DNA and to disturb normal cellular physiology and homeostatic control of tissue complicates investigations scrutinizing the interactions of UVR and the development of MM. However, human repair-deficient XP cells spontaneously undergo transformation in vitro only somewhat more readily than do normal human cells [288], and much less readily than rodent cells. This suggests that the difference in transformation efficiencies between human and rodent cells cannot be simply the increased capacity for DNA repair, but arise due to an innate difference in the development and retention of complementing mutations or other genetic perturbations. The development of cancer is a mutation-driven

process [289], but it is clear from patients predisposed to hereditary cancers [290,291] and from transgenic mouse studies [292,293] that simply harboring mutations in a particular cell lineage is insufficient for tumor development. Clearly, transformed or initiated melanocytes cells need the cooperation of a wide range of genes in order to progress. The crucial experiments are obviously to determine the precise nature of UVR targeted genes or gene elements, the timing of specific gene perturbations, the underlying biochemical malfunctions, the response of the host, and the repair of DNA in defined regions of the genome.

11. Summary and conclusions

Due to a variety of known and unknown control mechanisms, the human genome is remarkably stable when compared to most other species [294,295]. The long latency periods of most solid tumors, during which the cell undergoes malignant transformation, are presumably due to this stability. The molecular basis responsible for the induction of genetic instability and the resultant biological characteristics manifest in tumor populations is not well understood. The discovery of both oncogenes and tumor suppressor genes, however, has placed the phenomenon of human genome stability on a more solid conceptual footing. These types of genes clearly place multiple barriers to oncogenic transformation, and traversing these barriers apparently requires both time and the accumulation of genetic defects that cannot be corrected.

The evolution of neoplasias can, therefore, be predicted to be due to (1) consistent and progressive loss of tumor suppressor genes; (2) gene amplification, resulting in the over-expression of proteins that aid in tumor progression; (3) gene mutation, which alters the orderly biochemistry of the normal cell; (4) genes that allow a cell like the melanocyte to escape the confining nature of the epidermis and to invade through the dermis into the circulatory and lymphatic systems in order to disseminate itself to other organs (e.g., proteolytic enzymes, enzyme inhibitors, integrins, metastases genes, chemotactic factors etc.); (5) factors, perhaps such as TGFβ2, that may impact negatively on MHC antigens and confuse host defense mechanisms; and (6) S.O.S.-type genes, which may be expressed as a direct response to the accumulating damage in an attempt to correct the damage, but that may then become part of the problem instead of the solution.

The extraordinary plasticity and instability of the genome of a melanoma cell suggests an inordinate amount of genetic flux. In addition to activating and inactivating various genes, this constant shuffling and rearranging of the genome in neoplasms such as MM may be constantly altering gene dose. Cytogenetic and molecular biological studies have been the Rosetta stone for understanding the etiological relevant genetic events in human cancers [19]. Genetic alterations fundamental to the pathology of MM have begun

to be defined. Studies designed to understand these perturbations at the biochemical and organismic level are underway. Information about genes specific to the melanocyte and its transformed counterpart, the melanoma cell, can be used to great advantage, as recently shown by Smith et al. [296], who reported the detection of melanoma cells in peripheral blood by means of using PCR to screen for segments of the tyrosinase gene in patients with metastatic melanoma.

Clearly, exploitation of these types of observations could have great impact on the diagnosis and treatment of patients with melanoma, and any other cancer for which tissue-specific probes exist. The characterization of a gene or sets of genes whose expression or lack of expression may be capable of predicting, for example, if a primary tumor has metastatic potential and, if so, which organs are the likely targets of metastatic dissemination.

The definition of specific molecular and biological defects have already begun to provide multiple targets for diagnosis, for predicting clinical behavior, and for therapy. Figure 3 summarizes our current model of malignant progression of the human melanocyte. It incorporates information as to which chromosomes are perturbed and which specific genes are affected. As is evident even by a casual perusal of this figure, there are a number of genes known to be affected in primary melanomas of the vertical growth phase and in metastatic melanomas. Ongoing studies will soon determine if defects in these genes occur earlier in the transformation process. The locations of this set of affected genes are, for the most part, not on chromosomes believed to be involved early in the transformation process. Consequently, either these specific gene perturbations are late phenomena (being involved more in invasion and metastasis), or, more likely, their deregulation is caused by defects in other genes (e.g., transcriptional activators) that set in motion a vast array of alterations, not the least important being the activation of a gene(s) such as *ras*, which can destabilize normal chromosomal structure. Whether any of the critical genes on chromosomes known to be affected early in the transformation of the melanocyte are transcriptional activators or inducers of genetic instability remains to be determined. However, in due time it is anticipated that the combined efforts of numerous groups should elucidate the sequence of events necessary for the transformation and progression of the human melanocyte.

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9. Immunology and immunotherapy of melanoma

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1. Introduction

The successes achieved in the treatment of melanomas through immunotherapy suggest what may be possible in the treatment of other tumors. Although immunotherapy had to prove itself at first in patients with widespread tumors late in the course of the disease, melanoma patients have more recently been referred for treatment with adjunctive immunotherapy shortly after removal of their primary tumor and/or involved lymph nodes. Even patients with disseminated disease have been seen earlier than in the past, often before any chemotherapy has been administered, because chemotherapy for melanoma has been notably unsuccessful in prolonging useful survival despite increases in the rates of response.

There are many solid tumors, such as breast and ovarian cancers, for which chemotherapy is capable of inducing remissions with some frequency. Extension of survival has frequently not been a consequence of remissions, except for long-term complete responses. Even with those tumors that have not been treated preferentially with immunotherapy, where immunotherapy has been attempted there has in fact been some evidence for responsiveness [1,2]. Thus melanoma may prove to be not the exceptional immunologically responsive tumor, but simply one of the first tumors to exemplify what can be done to treat the whole gamut of human cancers.

In this chapter we will discuss several of the more successful types of immunotherapy for melanoma. Even though none by itself has caused responses in the majority of patients, several have given gratifying responses with increased survival in those who have responded. We will first describe some general aspects of tumor immunology and immunotherapy as a basis upon which to summarize some of the more promising clinical results with immunotherapy against melanoma.

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2. Melanoma antigens

Melanoma antigens have been defined mainly through the use of mouse monoclonal antibodies. These have defined a 97 kDa transferrin-receptor-like molecule on the plasma membrane [3], membrane gangliosides such as GD2 and GD3 [4], and a structural mucin with a 250 kDa proteoglycan core [5]. Whether any of these antigens are important immunogens in humans remains to be proved, and in fact there are clinical trials with vaccines made from each of these antigens in progress. Boon and his colleagues have cloned a gene defining an epitope ('MAGE-1') recognized by CD8 T cytotoxic cells (Tc) in an HLA-A1 context [6]. MAGE-1 is found on a proportion of, but not all, melanoma cells examined. This is the only reported example to date of a fully sequenced gene encoding an antigen immunogenic to humans. Our group has recently cloned a different gene, from which has been derived an immunogenic 17 amino acid peptide that stimulates T helper cells (Th) from melanoma patients [7]. The full sequence and HLA Class II restriction of the epitope have not been determined. However, the gene appears to be found predominantly in melanomas and not in normal tissues [8].

3. Cell-mediated immunity against melanoma

The major effector cells in the response to melanomas, and to tumors in general, appear to be thymus-derived small lymphocytes (T lymphocytes, T cells). Within this category are T helper cells (Th) and T cytotoxic cells (Tc). Th release lymphokines, which are soluble mediators, such as interleukins (IL) 2, 4, and 6, that influence other leukocytes, including Th, Tc, and macrophages. Tc also produce lymphokines that include tumor necrosis factor (TNF)-α and lymphotoxin (TNT-β), but may cause lysis of tumor cells through other as yet undefined mechanisms. In general, Th have a cluster designation (CD) of CD4, while Tc are generally CD8. Suppressor effector T cells (Ts) that can downregulate immune responses are CD8, and suppressor-inducer cells are CD4. In mice there are Th1 cells producing IL-2, TNF-α, and interferon (IFN)-gamma, leading to delayed-type hypersensitivity reactions useful against foreign cells, such as parasites. Th2 cells produce IL-4, IL-5, and IL-10, and lead to allergic responses characterized by eosinophilia. Preliminary evidence indicates that the same subcategories of Th may be present in humans. The distinctions between activities of CD4 and CD8 T cells have become somewhat blurred as each type has been examined more closely. Thus there are cytotoxic CD4 cells, and CD8 cells that can produce lymphokines, providing help in immunological reactions.

In melanoma specifically, we and others have demonstrated both CD8 and CD4 Tc in the blood of patients who are immunized with a melanoma therapeutic vaccine (theraccine) (J Kan-Mitchell, L LeMay, P Goedegebuure, W Harel, MS Mitchell, manuscripts submitted for publication; F Vanky,

personal communication, 1991) [9]. Clones of CD4 Tc have shown less intrinsic potency but the same sort of genetic restriction of their activity as their CD8 counterparts.

Both Th and Tc recognize antigenic determinants (epitopes) of melanomas as they are presented by major histocompatibility complex (MHC, HLA) molecules. CD4 Th recognize epitopes presented by Class II molecules on antigen-presenting cells (dendritic cells in the skin, macrophages in lymphoid tissues, and astrocytes in the brain) that have processed the 'foreign' antigens. CD8 Tc recognize epitopes presented by Class I molecules on the tumor cells themselves. Interestingly, CD4 Tc are Class I predominantly restricted too. That is perhaps not surprising, because endogenous epitopes, including tumor antigens, are primarily expressed by Class I molecules, and so Tc directed against them would have to be designed to recognize them in that context.

'Natural' killer (NK) cells, which are found in normal individuals as well as those with a tumor, may perhaps play a role in surveillance against tumors, and in some cases may participate in rejection. When activated by IL-2 or IL-4, NK cells and T cells can acquire a broader range of killing capacity against tumors but not normal cells. Lymphocytes with this activity have been called *lymphokine-activated killer cells* (LAK) [10].

Macrophages are probably the major effector cells in vivo, even though cytotoxicity by Tc can be shown in vitro. Macrophages predominate at the sites of rejection after treatment with theraccine, as they do in delayed-typed hypersensitivity reactions. In lesions biopsied after IL-2 and reinfusion of ex vivo-activated peripheral blood cells, lymphocytes (T cells) have predominated, often spread diffusely throughout [11]. This appears to be an exception to the general rule. Lymphokines from T cells, such as IFN-gamma, can cause macrophages to migrate to the site of a tumor and can activate them to become nonspecifically cytotoxic. In addition to their effector function, macrophages also make mediators, called monokines, such as IL-1 and TNF-α, both of which influence T cells. There are also suppressor macrophages that act in part through the release of prostaglandins of the E series. These may be important as suppressor effectors induced by products of the melanoma cells.

The coordinated activity of T cells and macrophages in vivo may account for the rejection of individual tumor nodules that have been observed after immunotherapy. We must stress, however, that the exact mechanism of rejection is unknown and may involve the release of cytokines, other than direct cytotoxicity by the cells or perhaps even ischemic necrosis caused by the perivascular accumulation of chronic inflammatory cells.

3.1. Role of antibodies in rejection

It is likely that complement-fixing antibodies do not play much of a role in rejecting tumors, because complement is not found in the interstitial spaces surrounding tumor cells. Antibodies that bind to tumor cells at their antigen-

combining sites in the Fab region, leaving their Fc region exposed and free to bind to Fc receptors on macrophages or activated NK cells, may be more important. These antibodies mediate antigen-dependent cell mediated cytotoxicity (ADCC). Whether they are important in the host's intrinsic response is uncertain, but it is likely the ADCC mechanism that accounts for whatever effectiveness has been found with monoclonal antibodies administered unconjugated to isotopes or toxins. MAb R24 against ganglioside GD3 also appears to attract Tc to the site of the tumor, although it does not act in ADCC with these Tc.

In our studies of active specific immunotherapy, we have consistently failed to find an association between the production of serum antibodies against melanoma cells (by enzyme immunoassay) and the response to therapy. In contrast, in each trial we have noted an association between the production of cell-mediated immunity, as measured by increased frequency of cytolytic T lymphocyte precursors in the blood, and clinical response.

3.2. Obstacles to the recognition of tumor-associated antigens by T cells

It is possible that tumors evade immunological rejection by failing to present their antigens in an optimally immunogenic form. Tumor antigens are considered to be weakly immunogenic, since they do not seem to differ greatly from those on their normal tissue counterparts, and because they are released in soluble form. The tumor-bearing host may have some degree of immunity against the tumor, but (demonstrably) not enough to reject it. If the tumor cells do not display their antigens, through a down regulation of the transcription or translation of genes encoding those antigens, even highly immunized cells cannot recognize and reject the tumor. The activity of suppressor factors from the tumor or suppressor elements they elicit may also hamper the immune response to the tumor.

These factors suggest strategies to use as part of a combination of agents for immunotherapy: (1) representation of tumor antigens to the host in a more immunogenic form, such as particles with an adjuvant to improve antigen processing; (2) additional stimulation of effector cells by cytokines; (3) inhibition of suppressor elements; and (4) upregulation of the expression of MHC and tumor antigens on the cell to permit optimal recognition by cytolytic effector T cells. Many of these strategies have already been used, singly or in combination, in clinical protocols for attacking melanomas.

With this brief immunological background, we will now discuss the specific results that have been obtained recently with immunotherapy for melanomas in the context of our categorization of immunotherapy in general.

4. Types of immunotherapy

The concept of biomodulation refers to any manipulation of the host-tumor relationship whereby the host is able to overcome the tumor [12]. This

subsumes the older category of 'immunotherapy,' yet it is still useful to consider the classical types of immunotherapy as a background for the rest of our discussion. Immunotherapy may be conveniently classified into five categories:

1. Active: Stimulation of the innate immune system of the host. This may be further subdivided into:
   a. Nonspecific: Activation of natural killer cells and macrophages, i.e., nonspecific effector cells, through the use of microbial or synthetic agents, also referred to as adjuvants. Examples of adjuvants include cell wall extracts of Mycobacteria, Salmonella, or Serratia species.
   b. Specific: TAA or tumor cells altered by mechanical methods, enzymes, or other processes to activate specific effector cells (cytotoxic T cells). Therapeutic vaccines (theraccines) are the models for this subgroup.
2. Adoptive: Transfer of effector cells and/or naturally occurring activating substances such as interleukins to the host in supraphysiologic amounts. Interleukin-2 with or without ex-vivo cultivated lymphokine-activated killer cells (LAK) falls into this group.
3. Restorative: Repletion of deficient host immune response. This is most often attempted by the inhibition or abrogation of host suppressor T cells through the use of low-dose cyclophosphamide or of suppressor macrophages with indomethacin or cimetidine.
4. Cytomodulatory: This newest category denotes the upregulation of major histocompatibility complex (MHC) or tumor-associated antigens (TAA) on the tumor cell surface, which improves the identification of the tumor cells by immune effector cells. Interferon-α, which has other properties that overlap into other categories, may have its most important effect through its alteration of tumor MHC antigens. Interferon-γ and tumor necrosis factor-α are other examples.
5. Passive: Transfer of exogenously produced antibodies or other antitumor factors to the host. This category includes monoclonal antibodies given separately or conjugated to other substances, including radioactive isotopes such as I-131, toxins such as ricin, or chemotherapy drugs.

5. Adoptive immunotherapy

Adoptive immunotherapy has attracted much attention in metastatic melanoma since Rosenberg and colleagues at the National Cancer Institute (NCI) first reported four responses (one complete and three partial) in seven patients [57% response rate (RR)] using bolus IL-2 + LAK cells [13]. IL-2 + LAK cell therapy caused five partial responses (PR) and two minor responses in 10 patients with a continuous infusion IL-2 schedule [14].

More definitive studies from the same groups subsequently reported lower response rates. Rosenberg et al. later showed two complete responses (CR) and four PR (RR = 23%) in 26 patients treated with the same IL-2 + LAK

regimen [15]. Dillman et al. described four PR in 33 additional patients (RR = 12%) with the continuous infusion regimen [16].

Because the potential toxicity of IL-2 + LAK therapy can be severe, specifically in regards to a 'capillary leak syndrome' with hypotension, oliguria, fluid retention, and possibly pulmonary edema requiring intubation [17–19], numerous studies have been performed in hopes of lessening toxicity as well as improving efficacy.

The Extramural Working Group (EWG) of the NCI treated 36 patients with the NCI bolus regimen [20]. One CR and five PR (RR = 19%) were seen, with the median duration of response being 5 months, although the CR and one PR were durable at 31+ and 13+ months, respectively. Responses occurred in subcutaneous tissue, lymph nodes, liver, and lung. No correlation could be found between either total IL-2 dose or the number of LAK cells infused and the response. One toxic death in a patient who experienced cardiac dysrhythmia and myocardial infarction occurred. Other significant toxicities included hypotension, requiring pressor agents in 24 patients (67%), and cardiovascular incidents, such as angina, dysrhythmia, or infarction in eight other patients.

The EWG also tested a combination bolus followed by a continuous infusion regimen in 50 patients [21]. One CR and six PR (14% RR) were seen. The median response duration was 21+ months, with responding sites including subcutaneous tissue, lymph nodes, liver, lung, and abdominal and pelvic masses. Significant treatment-related toxicities included hypotension, necessitating pressor support in 17 patients (34%); dyspnea at rest, but not requiring intubation, in six patients (12%); and hemodynamically significant cardiac dysrhythmia in three patients (6%). No therapy-related deaths occurred.

A continuous infusion regimen was found to be ineffective by the EWG [22]. Only 1 PR in 33 patients (3% RR) was seen, and there were four treatment-related deaths (three due to infection, one from hemorrhage). Other toxicities were frequent: 15 patients with hypotension requiring pressor agents (45%), 12 with dyspnea at rest (36%), 3 requiring intubation (9%), and 6 with cardiac dysrhythmia (18%).

Much evidence suggests that the addition of LAK cell infusions does not contribute significantly to melanoma therapy when compared to IL-2 alone. First, LAK cells are not specific for tumor cells but show some cytotoxicity for normal cells [23]. Lotze et al. have shown that LAK cells do not target selectively to metastatic sites [24]. Dutcher et al. showed that even though a greater number of viable LAK cells were infused into patients in the EWG continuous infusion study, the RR for this study was the lowest of all the EWG trials [22]. Similarly, other trials have found no correlation between number of LAK cells infused and the response in melanoma [25]. Both the NCI Surgical Branch and the EWG have shown no difference in RR between IL-2 alone and the IL-2 + LAK treatment arms [15,20–22,27]. Although there were more CR in the IL-2 + LAK arm in the NCI Surgical

Branch trial, other studies do not show as high a CR rate with infused LAK cells [26].

Parkinson and colleagues demonstrated that bolus IL-2 without LAK cells was active in melanoma [27]. Two CR and 8 PR were seen 46 patients (RR = 22%), with responding sites including subcutaneous tissue, lymph nodes, lung, and liver. The median duration of response was 8 months. Toxicity was similar to that described in studies that included LAK. Other groups using different IL-2 schedules without LAK have also demonstrated activity against melanoma [28,29].

In melanoma, in contrast to the case in renal cell cancer, it appears that the effects of IL-2 are mediated by T cells rather than LAK. In tissue culture, activated cytotoxic T cells have been shown to be significantly more cytolytic than LAK for melanoma cells [30,31]. Biopsies of melanoma have often revealed infiltration by T-lymphocytes, while LAK are few in number [32]. T cells are able to track specifically to metastatic sites, unlike LAK [33,34]. These reasons have generated considerable interest in the clinical application of cytotoxic T cells.

Tumor-infiltrating lymphocytes (TIL) may be separated from solid tumors such as melanoma, selectively expanded in culture, and when subsequently reinfused into patients, the TIL are able to home to sites of disease [35,36]. Rosenberg et al. reported a RR of 38% in 50 patients treated with cyclophosphamide 25 mg/kg IV on day 1 followed 24 hours later by TIL $1-3 \times 10^{11}$ cells then IL-2 720,000 U/kg every 8 hours [37].

Dillman et al. found a response rate of 24% using cyclophosphamide 1 g/m^2 IV day 1 followed by IL-2 18×10^6 IU/m^2/day via 96-hour continuous infusion with approximately 10^{11} TIL infused on day 2 [38]. Kradin et al., using a TIL + IL-2 regimen without cyclophosphamide, have demonstrated a 23% response rate [39]. The latest extension of this therapy is the recent development of gene-modified TIL [40]. One potential use for such TIL is to deliver cytomodulatory agents, such as tumor necrosis factor-α or interferon-γ, in high concentrations directly to targets, thereby reducing systemic toxicity and perhaps causing greater effects on tumor cells.

At present, though an interesting possibility, TIL + IL-2 appears to have practical difficulties, including (1) the need for accessible autologous tumor from which to obtain the TIL, (2) long incubation and growing periods required for TIL before any therapy is given, (3) inability to grow sufficient numbers of TIL in many patients, (4) possible heterogeneity of the TIL population, and (5) uncertain benefit of this therapy compared to other IL-2 regimens. All of these problems presently limit the applicability of this therapy [31,41].

As it is unclear whether the infusion of ex vivo activated cells (whether LAK or TIL) adds any benefit to IL-2 alone, and as high-dose IL-2 alone may have significant toxicity, another approach to adoptive therapy would be to attempt to utilize lower amounts of IL-2 and to attempt to benefit from the activity of cytotoxic T cells by selectively diminishing T-suppressor

cells with low-dose cyclophosphamide. Mitchell et al. have used a combination of low-dose cyclophosphamide and IL-2 to treat 39 patients, 10 of whom responded (26%) [12]. Two CR and eight PR were seen. Sites of response included liver, lung, lymph nodes, and subcutaneous tissue. Four responses (two CR, two PR) were seen in 10 patients with liver metastases. The median survival of responders was 18 months as compared to 8 months for the group as a whole. One 'minor response' (44% shrinkage of skin and lymph nodes) lasted for 4 years.

Toxicity was less than in high-dose IL-2 or IL-2 + LAK regimens. Although the toxicity commonly expected with IL-2, such as hypotension and dyspnea, occurred, the percentage of patients experiencing grade III/IV toxicity was much lower, 10% and 2%, respectively. Importantly, this therapy was administered on an outpatient basis rather than in an intensive care setting or on a medical ward, as in prior IL-2 studies.

In contrast, Lindemann and colleagues tested a cyclophosphamide + IL-2 schedule with a longer course plus dose escalation of IL-2 in 18 patients [42]. They showed an 11% partial response and a 4% minor response rate. Oldham et al., using a continuous infusion IL-2 schedule and a higher cyclophosphamide dose ($1\,g/m^2$) than in the previously mentioned studies, found no responses in eight patients [43]. It would thus seem that dose escalation of either IL-2 or cyclophosphamide does not improve the efficacy of this regimen.

Although there is some variability in response rates, the cyclophosphamide + IL-2 regimens compare favorably in efficacy to higher dose IL-2 regimens, but with the apparent advantages of overall less toxicity and possible outpatient administration.

6. Cytomodulatory immunotherapy

Alpha interferon has been tested in numerous doses and schedules in melanoma [44–46]. By itself it yields response rates of approximately 15%, which compares favorably with the most efficacious single cytotoxic agents [47]. The most common sites of response are subcutaneous, lymph nodes, and lung, although occasional responses in viscera are seen. A current intragroup cooperative study is underway to test alpha interferon in an adjuvant setting.

The activity of natural killer cells can be increased by interferon [48]. However, the usual doses found to elicit responses in most trials tend to be higher than those thought to enhance natural killer function [49]. Thus the mechanism of action of interferon may be more related to either a direct antiproliferative effect on melanoma cells [50] or to the augmentation of class I MHC antigens or TAA [51]. Interferon's cytomodulatory effect makes it appealing to use in combination with other biomodulators.

The most common toxicities of interferon include fever, chills, and

myalgia, which are frequently seen initially but diminish over the course of therapy [52]. Fatigue is often the dose-limiting toxicity [53]. Less common side effects include neurologic symptoms, such as confusion, rash, and infrequently leukopenia or thrombocytopenia [52].

Other cytomodulators, such as interferon-γ and tumor necrosis factor-α, have shown little activity as single agents [54–57].

7. Restorative immunotherapy

Some cytotoxic agents when used in less than maximally tolerated doses may have biomodulatory effects [58]. Cyclophosphamide has been shown in both animals and humans to have an augmentative effect on cell-mediated immunity [58–63]. This may be due to inhibiting the function or number of T-suppressor cells or precursors to T-suppressors or to the reduction of soluble factors (such as C-reactive protein) that have immunosuppressive effects [60,62,63]. This relatively selective effect on T-suppressor cells has led to the use of 'low-dose' cyclophosphamide, 350–500 mg/m^2, as part of a combination with other biomodulators, especially IL-2, as described previously [12].

Cimetidine has immunomodulatory effects, including increasing NK cell activity, increasing endogenous levels of IL-2, and inhibiting T-suppressor cells [65–67]. While occasional reports of PR in various tumors have been reported [67,68], other trials of cimetidine as a single agent in melanoma have shown little activity. Recently, Mandanas and colleagues found no objective responses in 15 patients who received cimetidine 600 mg qid [69].

Indomethacin and other nonsteroidal antiinflammatory drugs (NSAIDS) have displayed some activity in inhibiting suppressor macrophages [70,71]. Suppressor macrophages are thought to be able to diminish cell-mediated immunity to cancer through a prostaglandin-E-mediated mechanism that NSAIDs can inhibit [72]. One report has indicated that a combination of ranitidine and indomethacin was able to produced clinical responses in renal cancer [73].

8. Active immunotherapy

Specific acitve immunotherapy is typified by antitumor therapeutic vaccines (theraccines). Melanoma has been the most extensively studies tumor with this approach. Autologous preparations obtained from an individual patient, allogeneic formulations, and xenogeneic materials have all been tested. Even within a category, a direct comparison of the many different trials involving different theraccines is impossible. A variety of doses and schedules have been used, and often patients whose melanomas have ranged from

local or regionally resected to metastatic have been included within the same trial. Nevertheless, some representative studies can be summarized.

In an early trial, Laucius et al. treated 18 patients with irradiated autologous tumor cells ($1-2 \times 10^8$) and Bacillus Calmette-Guerin (BCG) intradermally in five divided doses every 2 weeks for five cycles [74]. While two CR and two PR were seen, the authors point out that these responses were short lived (duration of response = 2–4 months) and were limited solely to nodal and subcutaneous disease.

Murray and colleagues used weekly subcutaneous injections of melanoma cell oncolysate prepared with Newcastle disease virus in 13 patients [75]. Six patients showed some degree of shrinkage of skin nodules and/or lymph nodes; one CR was obtained with combination lysate and chemotherapy in a patient with lymph node involvement. No responses were seen with visceral disease. Bystryn et al. treated 13 patients with a polyvalent tumor antigen vaccine prepared from cultured melanoma cells [76]. One surgically assisted CR was seen in a patient with cutaneous metastases. There were no responses seen in visceral sites of disease.

Mittelman et al. conducted two clinical trials of the antiidiotypic monoclonal antibody MF11-30, which has the internal image of high molecular weight melanoma-associated antigen [77]. Thirty-five evaluable patients were treated with subcutaneous injections on days 0, 7, and 28. One CR in lymph nodes for 8 months and six minor responses were seen.

Berd and colleagues treated 76 patients every 28 days with cyclophosphamide 300 mg/m^2 on day 0 followed on day 3 by an autologous theracine comprised of $10-25 \times 10^6$ enzymatically dissociated and irradiated tumor cells with BCG as an adjuvant [78]. Twenty-four patients underwent resection of all known metastastic disease. Twelve patients were considered inevaluable because of rapid disease progression before the completion of 8 weeks of therapy. In 40 evaluable patients (having completed 8 weeks of therapy), four CR and one PR occurred (RR = 13%). Ten months was the median duration of response. Responses were seen in skin, subcutaneous sites, lymph nodes, lung, and possibly liver. Toxicity included mild nausea and/or vomiting, and local inflammatory changes, such as ulcerated papules and drainage of clear fluid at the injection sites.

Mitchell et al. treated 25 patients with a theraccine consisting of allogeneic melanoma lysates and an adjuvant (DETOX TM, RIBI ImmunoChem Research, Hamilton MT) derived from *Salmonella Minnesota* endotoxin and *Mycobacterium phlei* cell wall skeleton given on weeks 1–4 and 6 [79]. One CR and three PR (RR = 16%), and one long-term stability for 17 months (4%), were seen. Two mixed responses were also noted (8%). The median duration of response was 16 months. Sites of response were skin/subcutaneous tissue, lymph nodes, breast, and gut. Toxicity consisted of soreness at the injection site in most patients, inflamed granulomas in five patients, local abscess in one patient, and fever and malaise in one patient.

The most recent update of our melanoma theraccine experience at the

University of Southern California shows five CR and 15 PR (RR = 20%) in 106 total patients [80]. All of the responding patients were from the group whose CTL was elevated by the immunotherapy. Moreover, several HLA class I alleles — A2, B12, and C3 — were found to be highly associated with a limited response. In responders, the duration of survival is 31+ months, compared with 7.75 months in nonresponders, which is also the usual duration expected for metastic melanoma patients. The median duration of response is approximately 12 months. Sites of response now include lung and liver, in addition to those described previously. Toxicity remains minimal, with occasional palpable granuloma due to the adjuvant at injection sites. Virtually no systemic toxicity has been seen. The few cases of systemic toxicity appear to be related to prior exposure to BCG given for prophylaxis for tuberculosis. Patients receiving 15 or more injections have had sterile abscesses. Thus long-term responders have received melanoma lysates alone thereafter. These data suggest that T cells elicited by shared melanoma antigens in the theraccine could affect the rejection of autologous tumors in an optimal HLA context.

Numerous trials of melanoma vaccines have been conducted in the adjuvant setting [81–84]. Some have noted improved survival with vaccine therapy. However, these have generally been single institution studies using historical controls for comparison to establish efficacy. A multiinstitutional group trial randomizing patients to theraccine vs. observation alone is needed to provide more definitive information in this area.

9. Passive immunotherapy

Monoclonal antibodies (mAbs) are felt to have two major immunologic mechanisms of action. First, the variable region of the mAb binds to the tumor cell while the Fc receptor attaches to serum complement, which would then mediate disruption of tumor cell membrane. A second immunologic action of mAb can be through the binding of an effector cell, such as an NK/LAK or tumoricidal macrophage, to the Fc receptor of the antibody. An immune effector is thereby brought into intimate contact with the tumor cell, where it is subsequently able to lyse the tumor cell [85].

In therapeutic trials against melanoma, murine IgG3 mAbs to the disialogangliosidedel GD-3 (R-24) has been the most successful. Vadhan-Raj et al. used R-24 in 21 patients in a phase I trial and demonstrated a response rate of 19% [86]. Interestingly, three PR occurred on the two lowest of the four dose levels. Responding sites consisted of the skin, axilla, mediastinum, and lymph nodes. Response durations lasted from 2.5 to 11.5 months Toxicity was dose dependent and consisted of urticaria, pruritis, nausea and vomiting, and diarrhea.

Raymond and colleagues tested R-24 in a phase I trial by continuous infusion [87]. One CR and one PR out of 12 patients (17%) were seen.

Again, both responders were treated at the lowest dose level. Toxicity included a flulike syndrome, nausea/vomiting, pain at tumor sites, anemia, and peripheral edema.

Cheung et al. found two PR in nine patients (RR = 22%) with 3F8 mAbs. Responding sites were the skin, lymph nodes, liver, and bone. The duration of PR was 5.5 and 14+ months [88]. Toxicities included back and abdominal pain, urticaria, and diastolic hypertension.

Monoclonal antibodies have also been conjugated to radioisotopes such as I-131, cytotoxic chemicals, and toxins such as ricin [89–92] in attempts to improve the efficacy of this therapy and/or to reduce toxicity of the cytotoxic component alone. In general, however, the response rates obtained with these immunoconjugates have not been superior to those obtained with mAb alone. Some of the difficulties inherent to both unconjugated and conjugated mAbs include a relatively low degree of localization in target tissue, which may be due to multiple factors, such as crossreactivity with normal tissue, circulating antigen, removal by lymphoid organs, and heterogeneity of tumor; antigenic modulation by the target over time; lack of access to the tumor, such as with diminished blood flow to the tumor or residence of tumor cells in the sanctuary of the central nervous system; and development of human antimouse antibodies, which serve to limit repeated cycles [93].

In addition, some additional problems may arise due to the conjugate. These may include a difference between the in vitro and in vivo stability of the linkage between mAb and conjugate; inability of the conjugate to internalize; added toxicity of the conjugate; and difficulty in producing adequate quantities of the immunoconjugate [94].

10. Combination immunotherapy

A logical extension of single agent studies is the combination of biomodulators from different categories of immunotherapy into a regimen to attempt to achieve additive or synergistic effects on melanoma. Besides approaching tumor cells through different mechanisms, the use of combinations have the theoretical advantage of lessening toxicity due to any single agent. The previously described combination of low-dose cyclophosphamide and IL-2 is one example.

Based on animal data showing synergy between alpha interferon and IL-2 in pulmonary metastases from sarcomas [95], Rosenberg et al. tested this combination in a dose-escalation trial in melanoma [96]. They used IL-2 3–6 $\times 10^6$ U/m^2 IV every 8 hours with alpha interferon 3–6 $\times 10^6$ U/m^2 IV every 8 hours, both on days 1–5, with subsequent cycles repeated after 10 days of rest. The decision to utilize an intravenous route of interferon was based on preclinical studies and concern over possible erratic interferon absorption due to fluid retention secondary to IL-2. Three CR and 10 PR were seen in

39 evaluable patients (PR = 39%). However, in 23 patients who received >3 × 10^6 U/m^2 of IL-2, the response rate was 43%. Although there was a suggestion of a dose-response relationship, the small number of patients in each subgroup did not allow for statistical significance. Toxicities appeared to be similar to bolus IL-2 regimens with fluid retention, hypotension, and dyspnea.

Sznol et al. treated seven patients with IL-2, 0.4–0.8 mg/m^2, and alpha interferon, 3 MU/m^2, IV every 8 hours [97]. No response was seen. Oldham et al. treated 69 patients with IL-2 18 × 10^6 U/m^2 by continuous infusion for 108 hours, along with alpha interferon, 3 MU/m^2 SC, every other day during IL-2. Cycles were repeated after a 2-week rest [98]. One CR and six PR (RR = 11%) were seen, with a median duration of response of 2.1 months. Pichert et al. found two PR (duration of response = 1–7 months) in eight patients treated with IL-2, 3 MU/m^2, continuous infusion on days 1–4 and alpha interferon, 6 MU/m^2 SC, on days 1 and 4, given on alternate weeks [99]. Responses were seen in soft tissue, lymph nodes, liver, and lung.

Budd and colleagues recommended IL-2, 8 × 10^6 U/m^2 IV, with alpha interferon, 10 × 10^6 U/m^2 IM tiw, based on phase I results where both IL-2 and interferon were dose escalated [100]. In this study, two CR and three PR were seen in 17 patients (RR = 29%). The responses were durable, with the CR continuing at 772+ and 880+ days, and one PR ongoing at 564+ days. Responding sites were soft tissue, lung, and liver. No clear dose-response relationship could be drawn from this study; in fact, one CR occurred with as little as 6 × 10^6 U/m^2 of IL-2 tiw. Two other studies have also recommended 'low dose' IL-2 (5–6 × 10^6 U/m^2/day) when combined with interferon [101,102]. Atzpodien et al. treated eight patients with IL-2, 9 × 10^6 U/m^2 SC, every 12 hours for 2 days, followed by 1.8 × 10^6 U/m^2 bid 5 days per week for 6 weeks with interferon 5 million U/m^2 tiw [103]. One PR lasting 4+ months and three patients with stable disease (median 2.5 months) were seen. Importantly, toxicity was low in this 'home therapy.'

Hauschild and Christophers found one CR (lung) and two stable disease in 19 patients treated with subcutaneous IL-2 and interferon-α [104]. Only grade I-II toxicity was seen. Bajorin et al. combined IL-2, 1 × 10^6 units/m^2 IV, over 6 hours on days 1–5 and 8–12 with R-24 mAb at four dose levels [105]. One PR in soft tissue lasting 6 months and two minor responses were seen in 20 patients. The PR occurred at the highest dose level tested. No novel toxicities not previously documented with the agents separately were seen. In view of the PR at the highest level and because the R-24 doses in the study did not result in saturation of tumor antigen sites [86], an ongoing trial is being conducted at the University of Southern California combining escalating doses of R-24 with cyclophosphamide and IL-2. We have previously treated 14 patients with a regimen of cyclophosphamide, IL-2, and melanoma theraccine [12]. Two PR and two minor responses were seen. Central nervous system toxicity, including depression, mental lapse, and/or transient (<1 minute) loss of consciousness occurred in four patients.

An ongoing trial at the Norris Comprehensive Cancer Center, University of Southern California, involves the use of interferon-α-2b in patients previously treated with allogeneic melanoma theraccine. The results are preliminary, but eight PR in 18 patients (44% response rate) have been seen. The mechanism of action of this combination is unclear, but it is possible that the effect of cytotoxic T cells generated by immunization by the theraccine was potentiated by the induction of increased tumor antigen expression by interferon. This rationale is similar to that behind the use of IL-2 with alpha interferon; there is, however, seemingly less potential toxicity in the combination of allogeneic theraccine and interferon. Nevertheless, further studies are required to determine whether there is indeed a higher response rate with the sequential therapy than with interferon alone.

11. Chemoimmunotherapy

There are multiple theoretical advantages of the combination of cytotoxic agents and biomodulators. First, as described previously, there are biomodulatory properties of some agents such as cyclophosphamide. A host of other agents given at less than usual therapeutic doses, including mitomycin-C, which can augment LAK activity [106]; adriamycin, which is able to increase endogenous IL-2 production, to increase T-cell-mediated cytotoxicity, and to alter the fluidity of tumor cell membranes, thereby making them more susceptible to lysis [107–110,115]; and cisplatinum, which can potentiate tumoricidal macrophages and NK/LAK [111–113], have potential for use as biomodulators. A possible use for therapeutic doses of cytotoxic agents would be to 'make room' for the infusion of ex vivo activated effectors (LAK or TIL) [114]. This clearing might also trigger a compensatory release of cytokines from the stroma of lymphoid organs, thereby promoting the growth of the infused cells [115]. The use of therapeutic doses of chemotherapy may also serve to reduce tumor burden. With this in mind, it is important to note that Rinehart et al. showed that even large doses of agents such as cyclophosphamide, cisplatinum, and etoposide, which are not stem-cell damaging, do not prevent the subsequent generation of LAK or cytotoxic T cells by IL-2 [116]. This was corroborated by Redman et al. who also saw LAK generation after therapeutic-dose DTIC and cisplatinum [117]. Thus initial cytoreduction by chemotherapy would decrease the number of tumor cells that the immune effectors would need to lyse. Next, it is possible that the problem of drug resistance leading to treatment failure could be lessened by the sequential use of biomodulators, which would lack crossreactivity. Finally, the potential for nonoverlapping toxicity would allow for improved patient tolerance of these combination therapies [118].

Sznol et al. studied IL-2 by continuous infusion on days 0–5 and 11–16; low-dose cyclophosphamide and adriamycin on day 9; LAK infusion on days

11, 12, and 14; and alpha interferon-2a for nine doses in 40 evaluable patients [119]. They found eight PR, with response durations ranging from 2 to 26+ months (median 4 months). Significant toxicity appeared to be IL-2 dose related and included dyspnea/hypoxemia, intubation, hypotension requiring pressors, and granulocytopenia.

Flaherty and colleagues obtained a 38% RR (8% CR) using DTIC and cisplatinum on day 1 and IL-2 on days 12–16 and 19–23 of each 28-day cycle [120]. Responding sites included soft tissue, lymph nodes, lung, liver, spleen, and adrenal glands. Richards et al. found that 55% of patients with melanoma refractory to IL-2 were able to respond to a cytotoxic regimen of carmustine (BCNU), cisplatin, dacarbazine (DTIC), and tamoxifen [121]. Based on this apparent lack of crossreactivity, they designed a trial of BCNU on day 1, DTIC and cisplatinum on days 1–3 and 22–24, IL-2 and alpha interferon on days 4–8 and 17–21, and tamoxifen continuously [122]. After two cycles of therapy, a response rate of 57% (24% CR) was seen in 42 consecutive patients. Importantly, the median duration of the CR was beyond 9 months, and the median duration of the PR was beyond 7 months. These results compare favorably with the response duration of approximately 3 months for the same chemotherapy regimen alone [123]. Twenty-two patients experienced vitiligo-like depigmentation. Of these patients, 17 (77%) also had objective tumor shrinkage. Only three PR were seen in those patients who did not experience this. Thus, depigmentation correlated with tumor response ($p < 0.005$) [124]. This phenomenon, which is felt to be immune mediated, has also been noted by us, although extensive vitiligo is not an inescapable side effect of successful immunotherapy in our experience.

12. Conclusions

In recent years much effort has been placed in phase I and II trials to further the understanding of individual biomodulators. While no single biomodulator is effective in the majority of patients with melanoma, there appears to be great promise in the combination of different classes of biomodulators, possibly utilizing cytotoxic agents judiciously as well. It is crucial to note, however, that common guidelines used in chemotherapy do not translate easily into practice with biomodulators. The lack of a dose-response relationship with many biomodulators and the considerable duration of partial and minor responses, and even disease stabilization in select patients, are two such exceptions. Similarly, the immune system has such subtlety that the maximum tolerated doses of agents are often not those with optimal biomodulatory effects. Future studies must thus seek to understand the fundamental principles of tumor recognition and rejection, and to define and monitor the immunological effects of biomodulators. Those investigations will enable us to optimize their efficacy and to decrease their toxicity for patients.

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10. Positive and negative growth regulation in melanoma: Growth factors, intracellular signalling, and the cell cycle

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1. Introduction

The balance between positive and negative growth regulators plays an important role in the transformation of the melanocyte into the malignant melanoma cell. In positive growth regulation cells are recruited from a nonproliferating state to the actively proliferating cell cycle. Negative growth regulators cause cells to leave the proliferating state and enter one of several possible nonproliferating states. The concentration of extracellular growth factors and the interaction of the cell with the extracellular microenvironment are major factors in determining whether the cell will enter the proliferative cycle and divide. Growth regulation should be distinguished from regulation of the proliferative cell cycle. In vivo, the major controls of growth regulation are those processes that determine the fraction of cells in the proliferating cycle [1]. One respect in which melanoma cells differ from normal melanocytes is the shift of the balance from the nonproliferating phase to the actively proliferating cell-cycle phase. Normal melanocytes only rarely divide in vivo [2]. The transformation of the normal melanocyte to the malignant melanoma cell is accompanied by major changes in the expression of and response to both positive and negative growth regulators, and the intracellular pathways that mediate them.

The proliferative phase or cell cycle is conventionally divided into G1, S phase, G2, and mitosis (M). During G1 phase, the cells prepare for replication of nuclear DNA; during S phase, the DNA is replicated; during G2 phase, DNA is repaired and the cells prepare for mitosis (M), during which the cells separate the replicated genomes and divide. It was recognized during the 1970s that the major cell-cycle control point for somatic cells is in late G1 [1]. At this time, termed the *restriction point*, (R), cells either go on to complete the cell cycle or enter the nonproliferative phase. To pass the restriction point requires a high rate of protein synthesis. A second control point in the cell cycle occurs at the transition between G2 and mitosis. In the embryonic cell cycle this is the major control point. In somatic cells this checkpoint can be activated by DNA damage or incomplete DNA

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replication. Cells that have undergone DNA damage are delayed in G2 for DNA repair, since premature entrance into mitosis can be lethal.

The biochemical mechanisms underlying these cell-cycle controls have been investigated recently in an elegant series of experiments by a large number of investigators, working in systems from yeast to human tumor cells [1,3–5]. The major control appears to be exerted by *c*yclin-*d*ependent *k*inases (*cdk*). These kinases have regulatory subunits (cyclins) that are destroyed and synthesized each cell cycle. The transition from G2 to M is regulated by the *cdc2* kinase in a complex with cyclins A or B. The mechanism underlying the transition from G1 to S is less well understood, but involves at least two of these kinases, *cdc2* and *cdk2*, which form a complex with a variety of cyclins, whose role at the G1 to S phase transition is just now being defined.

In adult somatic tissue, most cells are not proliferating. Many are found in a reversible quiescent state, while expressing a differentiated function (e.g., melanocytes produce melanin, fibroblasts produce extracellular matrix, and peripheral lymphocytes express receptors for unique antigens). Somatic cells will return to the proliferative state when the local microenvironment changes, for example, by the introduction of growth factors, wounding, or presentation of a specific antigen. Others are found in a terminally differentiated or senescent state, and cannot reenter the proliferative cycle, even though they remain metabolically active. An example of this is the terminal differentiation of adult neurons. In addition, under certain circumstances some cells leave the proliferative cycle and enter a pathway that leads to programmed cell death or apoptosis. It is becoming clear that this latter pathway is an important component of overall growth regulation at the tissue level [6,7]. Entrance to each of these pathways is controlled by the expression of specific genes that are just beginning to be identified. The failure to express these genes blocks cells from entering that pathway. This, in turn, can tip the balance toward a higher fraction of cells in the proliferative cycle, a prerequisite for cancerous growth.

Figure 1 summarizes the above view of overall control of cell growth. Somatic cells usually leave the proliferating cell cycle with a G1 DNA content, although there are a few exceptions. Nonproliferating cells with a G1 DNA content are often referred to as G0 cells. Cells in a reversible quiescent state can be recruited into the proliferating cell cycle by the addition of growth factors or by altering the extracellular microenvironment. For normal melanocytes, the major mitogenic growth factor appears to be bFGF. In vitro, entrance into the reversible quiescent state can be induced by a withdrawal of growth factors or the addition of negative growth regulators, such as TGFβ or interferon. Entrance into the reversible quiescent state is accompanied by downregulation of protooncogene expression, such as *myc* and *fos*, and upregulation of genes required for the induction and maintenance of quiescence [8–10]. The senescent state occurs when a cell has exhausted its proliferative potential and is functionally defined by

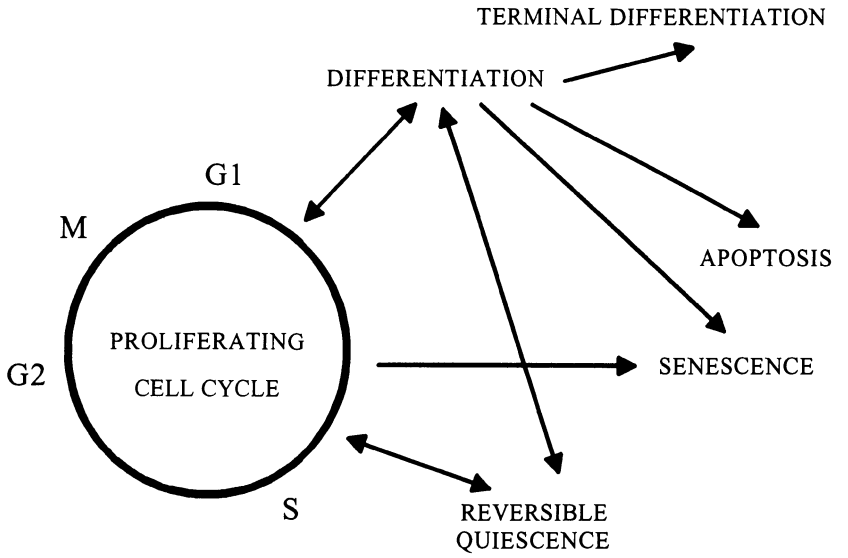

Figure 1. Growth control at the cellular level. Control of growth depends on regulation of cell proliferation, differentiation, and death. Differentiated cells are found both in the proliferative and quiescent populations. The regulation of the entry and exit of cells from the cell cycle is the major point at which the control of tissue growth is exerted. Cells can leave the proliferating cell cycle and enter a reversible quiescent state. Cells that do not die but cannot divide, and yet are metabolically active, are termed *senescent*. Several cell types undergo terminal differentiation and express their differentiated function but cannot be induced to divide. Programmed cell death or apoptosis is an important factor in determination of the rate of cellular proliferation. Proper balance of all of these states leads to orderly regulated growth.

failure of cells to reenter the proliferating cycle. It has been most extensively studied in fibroblasts, although normal melanocytes at high passage number do senesce [11]. Senescent human fibroblasts are unable to express protooncogenes that are usually induced when cells are recruited into the proliferative cycle [12]. Senescent fibroblasts also express specific cell proteins that are not found in proliferating or quiescent cells [13,14]. In order for a tumor to proliferate to the point when it would become a detectable mass, senescence must be delayed or prevented. The immortal phenotype, which is found in all tumorigenic cell lines, has been shown to be recessive [15]. Lastly, a large number of cell types appear to be able to undergo programmed cell death or apoptosis, which is characterized by a rapid and specific degradation of the cellular contents [6]. Apoptosis may be induced by chemotherapeutic agents [16] and, interestingly, may be controlled by oncogenes, including *bcl-2* [17,18] and *myc* [19]. It is important to note that even if the growth rate is constant, alteration of the death rate can change the net rate of tissue growth. Little is known, as yet, about the regulation of apoptosis in melanocytes and melanoma.

2. Regulators of melanoma and melanocyte growth

The requirements for growth of melanoma cells and normal melanocytes in vitro differ in two important respects. Melanocytes require exogenous fibroblast growth factor (bFGF) and 12-0-tetradecanoyl phorbol-13-acetate (TPA) for optimal growth, while melanoma cells require neither [20–22]. In fact, in most cases the growth of melanoma cells is inhibited by the addition of TPA [23]. Since these differences are observed in the vast majority of melanoma cell lines, they may be fundamental in the transformation of the normal melanocyte to the metastatic melanoma cell. Most melanoma cells grow well in tissue culture medium supplemented with fetal bovine serum, and many can grow readily in serum-free medium. In contrast, normal neonatal foreskin melanocytes require the addition of TPA. Eisinger and Marko [20] developed the first medium in which melanocytes could grow satisfactorily. This medium has been modified by others [11,24–26] but retains the essential components found by Eisinger and Marko. In this medium the cells maintain their differentiated phenotype, as seen by the elongated processes and the presence of pigmentation. In contrast, melanocytes grown in medium with bFGF, but without TPA, lose the elongated processes and appear much less differentiated [27] (our unpublished observations).

3. Role of bFGF

bFGF is a member of a family of at least seven related growth factors [28]. These include acidic FGF (aFGF), basic FGF (bFGF), KGF, HST, int-2, FGF-5, and FGF-6. There are several forms of bFGF that are observed in human cells. The predominant form, an 18 kDa monomer, is translated from an mRNA beginning at the AUG codon and is found in the cytoplasm, while other forms — 24, 22.5, and 22 kDa — are translated from upstream CUG codons [29–31]. Although bFGF is found extracellularly, there is no signal peptide, and it is not known how it gets out of the cell. Recently, the high molecular weight forms of bFGF, which have a nuclear localization signal, have been found in the nucleus, while the 18 kDa form is found primarily in the cytosol [31,32]. The intracellular location of the growth factor may be important in the execution of its function [33].

In vivo, melanocytes are found juxtaposed to keratinocytes and in close proximity to fibroblasts. It has been proposed that keratinocytes regulate the growth of melanocytes in vivo by providing bFGF [25]. Normal human fibroblasts also produce bFGF when grown in culture [34], but the level of bFGF mRNA depends on the growth conditions. Quiescent fibroblasts produce very low levels of bFGF mRNA, while stimulation by serum and other growth factors causes a major increase in its expression [34] (our unpublished observations). The normal melanocyte does not appear to produce bFGF or bFGF mRNA when grown in culture [34–37] (our un-

published observations). This has been established both by Northern blots and by the polymerase chain reaction (PCR) [37].

In contrast to normal melanocytes, exogenous bFGF is not required as a growth factor for melanoma cells. However, bFGF mRNA is produced by most melanomas [34–37] (our unpublished observations). This suggests that increased expression of bFGF may be a required step in the transformation of melanocytes. Support for this idea comes from two observations. First, most melanocytes with aberrant growth regulation, including common nevi, dysplastic nevi, and primary melanoma, express bFGF in vivo [38]. Second, an antisense approach was used to demonstrate that synthesis of bFGF was required for optimal growth of the malignant melanoma cells that had been adapted to serum-free culture. Most importantly, this inhibition could be abrogated by the addition of bFGF to the medium [39], suggesting that stimulation of the FGF receptor is still required for melanoma cell growth.

The fact that tumor cells expressed bFGF suggested that it might act like an oncogene when transfected into normal cells. When the cDNA for bovine bFGF was expressed in normal neonatal murine melanocytes, they showed increased growth and independence from exogenous TPA, but did not form tumors or express tyrosinase [27]. This transfection produced only the 18 kDa form of bFGF. In bovine aortic endothelial cells, expression of the high molecular weight forms of bFGF confers immortality, while the low molecular weight versions enabled them to grow in soft agar [40]. In NIH3T3 cells a high level of the high molecular weight form of bFGF is required for tumorigenesis [41]. Interestingly, if the bFGF cDNA is attached to a signal sequence, it is tumorigenic [42,43]. It should be noted that the cellular context in which bFGF expression takes place is important, since the ability to express high levels of bFGF is not sufficient, even for partial transformation, as shown by the example of normal human fibroblasts, which can produce a high level of bFGF, and its mRNA.

bFGF binds to high-affinity receptors that have tyrosine kinase activity [44] and also bind to heparin with low affinity, which is required for its optimal activity [45]. There are several types of FGF receptors (FGFR) with multiple and overlapping specificities for different members of the FGF family. The differing ligand specificity can be generated by alternative splicing and have distinct tissue distribution [46,47]. The cytoplasmic domain contains the tyrosine protein kinase activity and interacts with other cellular components, regulating the intracellular signalling pathways [48].

At the cellular level, bFGF appears to require the activation of protein kinase C (PKC) for full stimulation of mitogenesis [49–51]. However, the interaction between them is complex. In fetal bovine aortic endothelial cells, bFGF stimulated cell growth can be blocked by inhibition with the kinase inhibitor H-7 or downregulation of PKC up to the time at which the cells were entering the S phase. After this time the cells have completed the PKC-dependent events and proceed to divide [50]. This suggests that PKC may be involved in a critical event at or near the G1 restriction point. We consider the interaction between bFGF and PKC in more detail below.

4. Other growth factors

Although the effects of bFGF and regulation of its expression are the best characterized for melanoma cells and melanocytes, several other growth factors are expressed by melanoma cells. Platelet-derived growth factor (PDGF), transforming growth factor (TGF) α, TGFβ1, TGFβ2, and TGFβ3 are frequently expressed in melanomas [36,37]. In addition, other members of the bFGF family — aFGF, FGF-5, and KGF — have been found in some melanomas [37]. In contrast, melanocytes do not show frequent expression of aFGF , bFGF, TGFα, or TGFβ2. This suggests that an important step in the progression to melanoma is the expression of a variety of growth factors. With the exception of TGFβ, these growth factors are not expressed in normal human melanocytes [36,37].

As discussed above, melanoma cells have circumvented the requirement for exogenous bFGF by production of the growth factor. There are few other growth factor requirements for melanoma cells. The PG19 mouse melanoma line did not require the addition of insulin or insulin-like growth factor for growth in serum-free culture [52]; this contrasts with several human metastatic cell lines [36]. Human melanoma cells are not responsive (4 of 4 lines tested) to the mitogenic effects of PDGF or TGFα [36]. A survey of a large number of growth factors demonstrated that normal melanocytes were stimulated strongly only by members of the FGF family [53] and not by TGFα or epidermal growth factor (EGF) [11]. Normal human foreskin melanocytes are sensitive to the growth-inhibitory effects of TGFβ [11] (our unpublished results). Interestingly, many melanomas do not lose their sensitivity to TGFβ [36], although the growth of the Demel cell line is not inhibited by TGFβ (our unpublished results).

Recently hepatocyte growth factor (HGF) has been purified and cloned [54], and has been found to have the protooncogene c-*met* as a receptor [55]. HGF has specific receptors on human melanocytes and is mitogenic. It is also synergistic with both aFGF and bFGF [56]. Interestingly, it inhibits growth of at least one melanoma cell line [57]. Like the FGF receptors, the HGF receptor is a tyrosine kinase; however, the mechanism underlying its action has not yet been studied in detail. The regulation of melanocyte and melanoma proliferation by HGF has recently been reviewed [58]. It is possible that HGF may have an important role in differentially regulating the growth of normal melanocytes and metastatic melanoma cells.

5. Role of TPA and protein kinase C

Protein kinase C (PKC) was first discovered as a calcium- and phospholipid-dependent serine and threonine protein kinase. PKC is the cellular binding site for tumor-promoting phorbol esters, such as TPA. Four isozymes were discovered and characterized (α, βI, βII, and γ) [59]. More recently several

additional isozymes have been found by DNA homology, including δ, ζ, ε, and η [60–62]. These isotypes are not calcium dependent. All isotypes of PKC include a catalytic and an activation domain. In vivo, the enzyme is activated by diacylglycerol, which can be mimicked by the tumor promoter TPA. Activation by TPA leads to translocation of the enzyme from the cytosol to the particulate fraction and the subsequent proteolytic cleavage and downregulation of PKC activity [59].

As noted above, addition of TPA to the culture medium is required for the optimal growth of neonatal human melanocytes. However, the mechanism by which it stimulates melanocyte growth is not clear. In contrast, TPA inhibits the growth of most melanoma cells. TPA inhibits the growth of a large number of types of cells [63]. The mechanism of this growth inhibition is also not known in these systems. We have begun to analyze the mechanisms underlying inhibition of growth by TPA in the Demel melanoma cell line. Our studies have demonstrated that there is a direct effect on the cell cycle.

The acquisition of independence from exogenous bFGF and the coincident development of inhibition of growth by tumor-promoting phorbol esters strongly suggests that there may be a link between the two events. The activation of PKC must endure for an extended time in order to be effective [64–66], suggesting that multiple PKC-dependent events must occur before cells are committed to a specific pathway. In Swiss 3T3 cells, bFGF stimulates PKC activity and the phosphorylation of a major PKC substrate, p80, but not mobilization of Ca^{2+} [49]. In neonatal bovine artery endothelial cells, both bFGF and TPA are mitogens. bFGF has been shown to depend on PKC for its mitogenic activity in this system [50]. However, in the presence of continued stimulation by bFGF (30 ng/ml), TPA is actually growth inhibitory [66]. In addition, in a clone of NIH 3T3 cells TPA blocked bFGF-induced mitogenesis [67]. Hence, one possibility that must be considered is that when stimulation of the bFGF intracellular signalling occurs the cell becomes susceptible to growth inhibition by activators of PKC because of 'interference' or 'crosstalk' in the growth-stimulatory pathways. This inhibition may be dependent on the specific isozymes of PKC and the specific cellular context in which they are expressed. Interestingly, transfection of rat fibroblasts with PKCβI makes them more sensitive to growth factors [68], while TPA inhibits the growth of colon carcinoma cells that express a high level of transfected PKCβI [69].

In a nontumorigenic immortalized murine melanocyte cell line, MelAb, which depends on TPA for growth, proliferation was associated with low level of calcium-dependent PKC activity, low levels of phorbol ester binding, and low levels of PKC protein. The antibody used recognized both PKCα and PKCβ, but individual isotypes were not distinguished [70]. This suggests that TPA could be acting through the downregulation of PKC. In a recent study, Arita et al. [71] have demonstrated that human melanocytes contained the α, β, and ε subtypes and that β, but not α, was rapidly downregulated in response to exposure to high concentrations of TPA. However, concentrations

of TPA that were optimally growth stimulatory did not downregulate PKCβ.

The large number of isozymes of PKC makes it difficult to prove that the TPA-induced effects on cell growth regulation are mediated by either activation or downregulation of PKC. Detailed studies that analyze the role of each of these isozymes will be required to unravel the mechanisms by which TPA stimulates the growth of melanocytes, inhibits the growth of melanoma cells, and how each interacts with the pathways stimulated by other growth factors and oncogenes.

6. Effects of TPA on the melanoma cell cycle

Although TPA has been known to inhibit cellular growth in a large number of systems, the mechanism by which the addition of phorbol esters inhibits growth is not yet understood. One approach to this problem is to understand exactly what happens when the cells arrest growth and to work back to the events that can be directly tied to the application of TPA. Since the addition of TPA causes growth arrest, we examined how the melanoma cells arrested growth in the cell cycle. After a logarithmically growing population of Demel melanoma cells was treated with TPA for 24 hours, examination with flow cytometry demonstrated that there was a large G1 and G2/M population, but very few cells were found in S phase [23,72]. This is consistent with growth arrest in G1 and an arrest in either G2 or mitosis. Cells in S phase are able to complete DNA replication and to enter G2/M. The second arrest point appears to be in G2 rather than in mitosis. We have examined populations of cells that have been treated with TPA and that have more than 50% of the cells with G2 DNA content, yet only a few (2–4%) are found in one of the stages of mitosis.

To determine when the G1 arrest point occurred, we needed to have a synchronous population in G1 prior to the TPA induced arrest point. We have used lovastatin, an inhibitor of hydroxy methyl glutaryl CoA reductase (HMGCoA reductase), to synchronize the Demel cells in G1. Lovastatin acts to block the cell cycle by preventing the isoprenylation of a variety of proteins [73]. Two studies using monoclonal antibodies and flow cytometry have demonstrated that this arrest point is in the middle of the G1 phase [74,75]. However, the specific target of the growth-regulating isoprenylation is not as yet known, but probably is not the *ras* protooncogene [76]. Demel cells released from the lovastatin block by the addition of mevalonate enter S phase after 8–10 hours. If TPA is administered up to this point, cells do not enter S phase, suggesting that the TPA arrest point is very close to the time at which the commitment is made to enter S phase. If TPA is added after this time, an increasing proportion of cells enter S phase [72]. This suggests that pathways involving PKC are interacting with the cell-cycle control pathways, which are regulated by the cyclin-dependent kinases.

7. Role of cell cycle kinases in growth control

The study of regulation of the mammalian cell cycle has been illuminated by studies in many organisms, including yeast, Drosophila, clams, and frogs. The names of the genes that are important in the cell cycle reflect the way in which the genes were first discovered. There are several excellent reviews of the molecular basis of cell-cycle control to which the reader is referred for more detail [1,3–5]. Here, we wish to highlight those aspects that have direct relevance for the regulation of the growth of melanoma cells. In all eukaryotes, the cell-cycle kinase $p34^{cdc2}$ has a critical role in regulating the major control points of the cell cycle. In higher eukaryotes a related enzyme, *cdk2*, has an important role at the G1/S border [77,78].

In the fission yeast *Schizosaccharomyces pombe*, *cdc2* was identified as a temperature-sensitive mutant that arrested growth at the G1/S border and in G2 at the nonpermissive temperature. Like Demel melanoma cells that have been treated with TPA [72], the *cdc2* mutants show a biphasic distribution of cells in G1 and G2 [79]. However, there is no evidence suggesting that PKC directly modifies the $p34^{cdc2}$ protein. $p34^{cdc2}$ is found as a component of a complex that has histone H1 kinase activity and can induce the maturation of frog oocytes. These activities were purified and shown to consist of a kinase ($p34^{cdc2}$) and a regulatory subunit (cyclin) [80]. The kinase itself was found to be phosphorylated on at least three residues. These phosphorylations regulate the activity of the kinase at the transition from G2 to mitosis [4,81]. In Figure 2A, a diagram is shown outlining the important features of the structure of the $p34^{cdc2}$ protein and the control of the regulatory phosphorylations.

8. Regulation of $p34^{cdc2}$ at G2/M transition

The control of activation of the $p34^{cdc2}$ kinase and its functional role is best understood at the G2/M transition. In G2, the kinase is inactive and is phosphorylated on thr 14, tyr 15, and probably thr 161, and is found in a complex with activating subunits, cyclin A or cyclin B. In mitosis, the kinase is active and is dephosphorylated on thr 14 and tyr 15, is phosphorylated at thr 161, and remains in the complex with the cyclins. As cells leave M, the cyclins are degraded and the $p34^{cdc2}$ becomes dephosphorylated at thr 161. Binding of the cyclin appears to be required for phosphorylations. This sequence of events is diagrammed in Figure 2B.

Regulation of the arrest G2 induced by TPA in melanoma cells might be mediated at the level of any of these events. This is indicated by the dashed lines in Figure 2A. The tyr 15 is phosphorylated by the human homolog of the dual-specificity kinases, *mikl* or *weel*, originally discovered in yeast [82,83]. Interestingly, the cellular response to DNA damage, which causes cells to transiently arrest in G2, appears to be mediated through this pathway

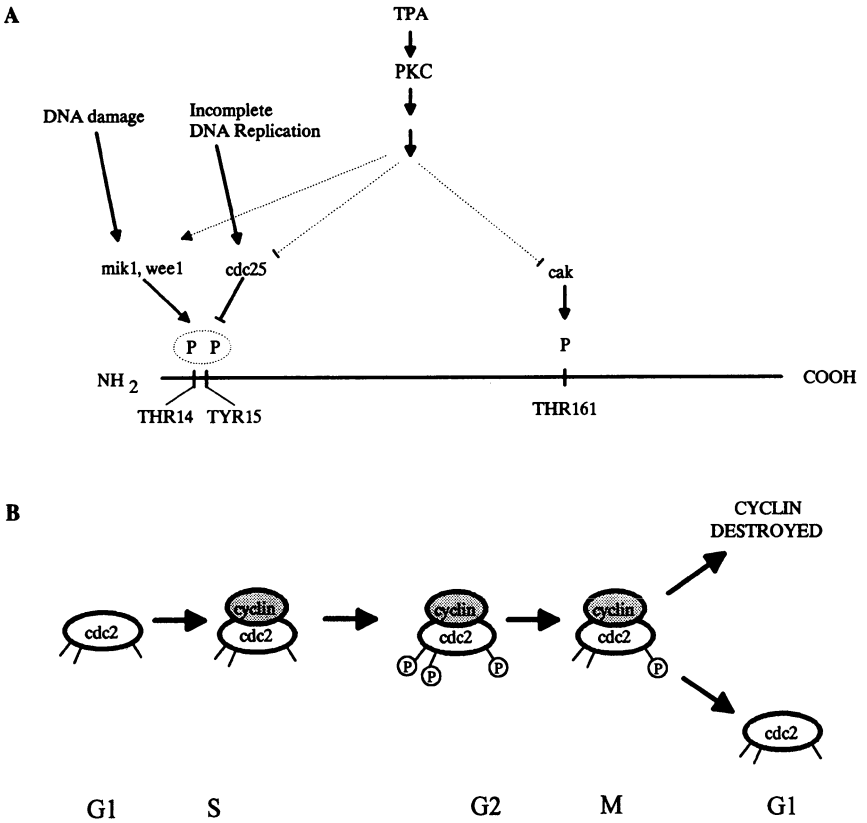

Figure 2. A: Structure of the cell-cycle regulatory kinase $p34^{cdc2}$. $p34^{cdc2}$ is the catalytic subunit of the histone H1 protein kinase. The activity of the kinase rises dramatically in the transition from G2 to mitosis. The activity of the kinase requires binding of cyclin and is regulated by phosphorylation of three residues, thr 14, tyr 15, and thr 161. Phosphorylation of thr 14 and tyr 15 is associated with inactivation of the kinase, while phosphorylation of thr 161 is associated with activation of the kinase. Tyr 15 is phosphorylated by the *wee1*-like kinases. These same residues are dephosphorylated by a *cdc25*-like kinase. If the timing of this phosphorylation is delayed, the cell will not enter mitosis at the correct time. The G2 delay induced by DNA damage may be mediated through the *wee1* pathway [84], while the G2 delay induced by incomplete DNA replication may be mediated through the *cdc25* pathway [81,86]. Phosphorylation of thr 161 is required for an active H1 kinase. A new kinase has been identified, a $p34^{cdc2}$ activating kinase (CAK) [87]. The dotted lines indicate *potential* sites of interaction between the PKC pathway and the *cdc2* pathway. The arrowheads indicate stimulation and the flat bars indicate inhibition. B: Activation of $p34^{cdc2}$ in the cell cycle. $p34^{cdc2}$ is inactive and exists as a monomer in G1. When cyclin is synthesized, it binds to $p34^{cdc2}$, forming a complex. During S phase and G2, $p34^{cdc2}$ is phosphorylated at three sites: thr 14, tyr 15, and thr 161. It is not active as a kinase in this state. At the G2/M boundary, thr 14 and tyr 15 are dephosphorylated, the kinase is activated, and the cells enter mitosis. Mitosis concludes as the cyclin is degraded and the $p34^{cdc2}$ is dephosphorylated to its basal state as found in G1.

[84]. The dephosphorylation of tyr 15 is controlled by a protein phosphatase, *cdc25*, which can dephosphorylate proteins phosphorylated on tyrosine [85]. Regulation of this phosphatase is critical for proper entry into mitosis. The feedback loop that prevents the entry into mitosis until DNA replication is complete is partially regulated through the regulation of the tyr 15 phosphate [81,86].

A third level of control is found in the regulation of the phosphorylation of thr 161. This residue must be phosphorylated for the activation of the $p34^{cdc2}$ kinase, permitting cells to enter mitosis. Recently a new kinase has been identified that regulates this phosphorylation [87]. A serine/threonine protein phosphatase, INH, has been identified that prevents premature entrance into mitosis and may regulate the dephosphorylation of thr 161 [88]. The network of kinases and phosphatases that regulate activation of the $p34^{cdc2}$ kinase and control entrance into mitosis show distinct levels at which TPA could lead to a G2 arrest in melanoma cells. These are shown as dashed lines in Figure 2A. The regulation of phosphorylation of $p34^{cdc2}$ as cells pass through the cell cycle is shown in Figure 2B.

9. The G1/S transition

The G1/S transition is comprised of two major regulatory events: (1) the commitment to a new round of cell division and (2) the onset of DNA synthesis. As noted above, commitment to the proliferative cell cycle requires a high rate of DNA synthesis, suggesting involvement of a rapidly degraded labile protein [1]. One possibility is that one of the short-lived cyclins may be such a protein.

It is now clear that there is more than one kinase that acts at the G1/S transition in higher eukaryotes [77,89,90]. The specific functions of the newly discovered *cdk2* kinase [78,91,92] and the *cdc2* kinase are still being worked out. Although $p34^{cdc2}$ clearly has a role in the G1/S transition for the yeast *S. pombe*, evidence for its role at the G1/S border in human cells has accumulated more slowly. A role for the $p34^{cdc2}$ kinase has been suggested in experiments designed to isolate the components required for the onset of DNA synthesis [93]. A recent report suggests that *cdk2* may have the dominant role in the G1/S transition in the embryonic cell cycle [77]. At least four cyclins, including cyclin A and the three 'G1 cyclins' — cyclin C, cyclin D, and cyclin E — have roles in proper regulation of the G1/S transition [94,95]. Interestingly, one of the G1 cyclins, cyclin D1, was found to be amplified in a benign parathyroid tumor [96].

There are several points at which TPA could act to inhibit progression from G1 into S phase. This could be at the level of synthesis of any of cyclins or kinases or at the level of posttranslational modifications of the kinases that are required for activation. We have begun to examine how the addition of TPA alters the synthesis and posttranslational modification of

one of these kinases, $p34^{cdc2}$. The $p34^{cdc2}$ kinase is phosphorylated at a very low level in the G1 phase. As cells enter the S phase, $p34^{cdc2}$ becomes phosphorylated and the level of phosphorylation remains high until the end of mitosis. In most systems the amount of $p34^{cdc2}$ protein is stable; however, a new round of synthesis begins as the cells enter S phase [97].

10. Regulation of $p34^{cdc2}$ at G1/S boundary in melanoma cells

In order to analyze how TPA inhibits the onset of DNA synthesis in Demel melanoma cells, we have begun to analyze the regulation of the phosphorylation and the synthesis of $p34^{cdc2}$ in cells that have been treated with TPA at specific times in the cell cycle. We analyzed the phosphorylation state through the cell cycle and found low levels of phosphorylation in G1 and high levels in S and G2/M cells. When TPA was added in G1, 8 hours after release from the cell-cycle block, it prevented this rise in $p34^{cdc2}$ phosphorylation [72].

Similarly, when we analyzed the synthesis of $p34^{cdc2}$ by immunoprecipitation of $p34^{cdc2}$ from cells metabolically labelled with ^{35}S-methionine,

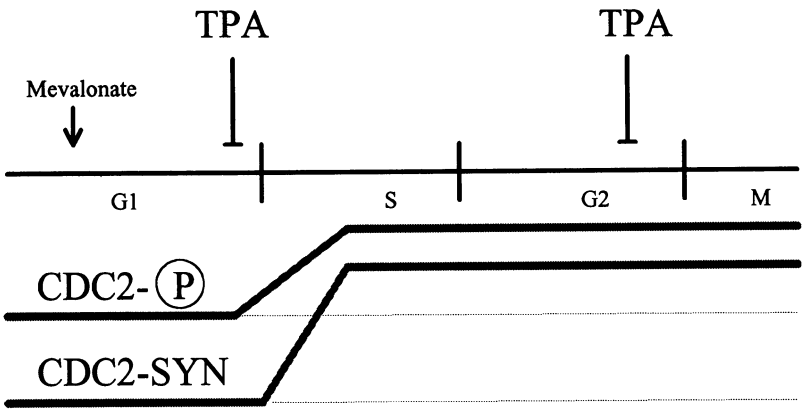

Figure 3. Model of TPA action at the G1/S boundary in melanoma cells. Cells synchronized in G1 with lovastatin are about 8 hours from the G1/S boundary. They have a low level of phosphorylated $p34^{cdc2}$ and also synthesize it at a low rate. They will progress towards the S phase when the lovastatin block is lifted by the addition of mevalonate. As the cells enter S phase, they increase the rate at which $p34^{cdc2}$ is phosphorylated and synthesized. By mid-S phase a maximal level is reached, and this is maintained through the rest of the cell cycle. If TPA is added before the G1/S boundary, it will prevent cells from entering S phase. If it is added after this point but before the G2/M boundary, the cells will arrest in G2. Addition of TPA before the G1/S boundary prevents the increased phosphorylation and synthesis of $p34^{cdc2}$. However, addition of TPA to S-phase cells does not reverse the high level of phosphorylation and synthesis, even though the cells arrest in G2.

we found that addition of TPA at the end of G1 phase inhibited the new synthesis of $p34^{cdc2}$ [98]. We are currently investigating if this is regulated at the level of synthesis of the $p34^{cdc2}$ mRNA or at the level of translation. In contrast, addition of TPA in mid-S phase blocked neither the synthesis nor the phosphorylation of $p34^{cdc2}$. This suggests that there is an important switch at the end of G1 that can be inactivated by the addition of TPA to melanoma cells. A model of these events is shown in Figure 3. In G1, $p34^{cdc2}$ is both phosphorylated and synthesized at a low level, while in S-phase, G2, and M, the level of phosphorylation and the rate of synthesis are high. Both remain high 24 hours after release from the G1 block, when most cells are found in late S phase, G2, and M. The addition of TPA blocks this transition.

These findings suggest that there is an important regulatory point in late G1 where the cell-cycle regulatory kinases interact with the intracellular signalling pathways. In mink lung epidermal cells, which are exquisitely sensitive to the growth inhibitory effects of TGFβ, the addition of TGFβ up to the G1/S border blocks the onset of DNA synthesis. Examination of $p34^{cdc2}$ demonstrated that synthesis and phosphorylation could be blocked at this point [99]. In the human B-lymphoblastoid Daudi cell line, both TPA and interferon-α block cell-cycle progression at the G1/S boundary and also prevent the phosphorylation and synthesis of $p34^{cdc2}$ [100]. Since TGFβ, interferon-α, and TPA all appear to induce the arrest of growth at the same point in late G1, it will be of great importance for future therapy to determine whether they all act through the same pathways.

11. Future directions

Transformation from the normal melanocyte to the metastatic melanoma cell involves tipping the balance of normal growth regulation toward less well-regulated growth. Understanding how the growth-regulatory networks of the normal melanocytes change during the transformation into the malignant melanoma cells requires a detailed examination of the pathways involving three major networks of protein kinases. The bFGF receptor is a tyrosine kinase and triggers a series of events leading to mitogenesis, including activation of PKC. The PKC pathway is involved in many types of intracellular signalling, besides activation or inhibition of mitogenesis. It will be necessary to determine the role of specific isozymes of PKC in melanocytes and melanoma cells, and to determine how these affect the proliferative response in the two cell types. Lastly, it will be important to determine at which level PKC interacts with the cell-cycle pathway regulated by the cyclins and *cdk* growth-regulatory kinases. Understanding the mechanisms underlying the changes regulating melanoma cell growth may lead to the design of new therapies that exploit these processes.

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11. The role of surgery in cutaneous malignant melanoma

Daniel G. Coit

1. Introduction

Although cutaneous malignant melanoma comprises only 3% of all cancers, its incidence is rising more rapidly than any other malignancy, increasing 83% between 1973 and 1987. If this trend continues, it is estimated that the lifetime risk of developing melanoma for someone born in 1950, 1:600, will increase to 1:90 for someone born in the year 2000. Fortunately, of the 32,000 new cases diagnosed in the United States in 1992, the vast majority will be diagnosed as a surgically curable stage. Between 1981 and 1987, it is estimated that 80% of all new melanomas were diagnosed as localized disease, with another 8% being diagnosed with locoregional disease only [1]. Even at a tertiary referral center, the majority of cases are diagnosed at a stage when surgery represents the mainstay of therapy (Table 1). Thus, it is imperative that we have a complete and thorough understanding of the surgical management of these patients at all stages.

Only by adequate recognition and appropriate biopsy of pigmented skin lesions can melanoma be diagnosed at an early, curable stage. A thorough understanding of the biology of the primary tumor, as well as data from recent prospective clinical trials, has prompted a more rational policy regarding the technique of wide excision, with attention to surgical margins that will both minimize local recurrence, as well as enhance the ultimate cosmetic result. The management of clinically negative regional lymph nodes in patients at risk for occult metastases remains controversial; data emerging from maturing prospective trials should clarify this in the next few years. Therapeutic lymphadenectomy clearly has a place in the management of melanoma patients, as a significant minority of patients with clinically positive nodes will be cured by lymphadenectomy alone. These are procedures that carry with them some morbidity, and there are a number of factors relating to the technique and extent of lymphadenectomy that need to be understood, again, to maximize locoregional control and survival while minimizing complications. Finally, even for patients with systemic dissemination of their melanoma, there is a role for resection. Proper selection of patients for

L. Nathanson (ed.), Current Research and Clinical Management of Melanoma.

Table 1. Pathologic stage at presentation of patients with cutaneous melanoma: Memorial Sloan-Kettering Cancer Center, 10/85–12/90

Stage	Number	%
Localized disease		
<1.5 mm	468	29
>1.5 mm	277	17
Regional disease	351	22
Systemic disease	523	32
Total	1619	100

surgical intervention mandates a thorough understanding of the natural history of melanoma metastatic to various sites.

In this review we will attempt to address many of these issues as they pertain to the clinician in an effort to arrive at a rational policy for the surgical management of patients with malignant melanoma.

2. Surgery for the primary tumor

2.1. Biopsy techniques

A modest controversy exists as to how best to establish the histologic diagnosis of primary melanoma, specifically whether to proceed with incisional or excisional biopsy. In many European centers, standard management would consist of definitive wide excision after the clinical, but not histologic, diagnosis of melanoma had been entertained. Rampen looked at 76 patients with pathologically confirmed local melanoma only, 14 of whom underwent incisional biopsy prior to definitive management [2]. The remainder underwent either wide excision or local excision followed by wide excision. He found that those patients undergoing incisional biopsy of their melanoma had a relative risk of dying 4.7 times higher than those undergoing either local excision or wide excision as their initial procedure ($p = 0.006$ by multivariate analysis). Of interest, in his small series analyzing 12 prognostic variables, the only other variables found to be predictive of poor survival included ulceration of the primary, age over 50, and male sex. Primary tumor thickness did not correlate with survival in his series.

Bagley, on the other hand, noted no difference in either the incidence of local recurrence or survival when comparing 22 patients undergoing incisional biopsy for primary melanoma to 125 patients undergoing local excision as their initial procedure [2].

Both Griffiths and Lees reported on the outcome of patient groups undergoing incisional biopsy, local excision, or definitive primary wide excision as their initial procedure [3,4]. While both authors noted no difference in

either local recurrence or overall survival, they did comment that incisional biopsy resulted in incomplete assessment of the primary lesion in 33–44% of patients. This compared to only 0.5–5% of lesions being technically unsuitable for staging after either local excision or wide excision.

Finally, Landtheler and Lederman, in separate reviews, compared the outcome of patients undergoing initial definitive wide excision with those undergoing lesser procedures, either incisional or punch biopsies, or local excision [5,6]. Both series found identical local recurrence and survival rates when comparing the two groups of patients. Landtheler, furthermore, commented that the time interval from biopsy to definitive management of the melanoma had no influence on the outcome. From the above it would appear prudent that, where technically possible, excisional biopsy of the primary lesion can safely be performed in order to obtain the relevant pathologic information for subsequent treatment planning.

There are clearly specialized instances in which excisional biopsy would interfere with definitive management of the primary, in which case incisional or punch biopsy would be sufficient. These might include very large primary lesions, lentigo maligna, lesions on the face where local skin flaps would be planned to cover surgical defects, subungual lesions, and ear lesions. In these instances, a biopsy of the grossly thickest portion of the lesion should be obtained to derive the most prognostic information possible.

Finally, it must be emphasized that all biopsies should be done with definitive treatment in mind. Specifically, biopsies of lesions on the extremity should be oriented along the axis of the extremity rather than transversely. This can very often preclude the necessity for split-thickness skin grafting. Incisional biopsy should not be placed in such a manner as to interfere with subsequent planned rotation flaps.

2.2. Management of the underlying fascia

Surgical practice varies with regard to whether or not the underlying muscle fascia should be included with the wide excision. Olsen observed that the subdermal lymphatics anastomosed with subfascial lymphatics. Despite this observation, she found that 14 of 31 patients (45%) who had their fascia removed during wide excision of the primary lesion subsequently developed regional metastatic disease, compared to only 5 of 36 patients (14%) of those patients whose fascia was left intact. There was no difference in the rate of local recurrence. This observation suggested that, at best, removal of the underlying muscle fascia did not enhance local control [7].

Kenady found an identical recurrence rate comparing 107 patients in whom muscle fascia was removed to 95 patients in whom the muscle fascia was preserved. Patterns of recurrence in overall survival were identical in both groups [8]. Thus it does not appear that removal of the muscle fascia is necessary in the management of primary malignant melanoma, and may in fact result in unsightly and unnecessary herniation of the underlying muscle.

2.3. Margins

Classical surgical teaching has emphasized wide surgical margins in the management of primary malignant melanoma. The origin of this philosophy is often attributed to Sir William Handley, whose 1907 Hunterian lecture described the lymphatic permeation of melanoma in the autopsy specimen of a 34-year-old woman who died of disseminated melanoma. In his presentation, he advised removing 'about one inch' of skin around the primary melanoma, then undermining the skin to remove 'about two inches' of subcutaneous tissue to maximize the chance of resecting all gross disease [9,10]. His recommendation was bolstered by the observations of Cochran and Wong, both of whom observed abnormal melanocytes within 5 cm of certain primary melanomas [11,12].

More recent series have refined these observations. Microscopic satellitosis has been observed, more often in association with thicker primary melanomas. Elder found histologic satellitosis in 5 of 23 patients (22%) with melanomas thicker than 2.25 mm, compared to 0 of 82 patients with melanomas <2.25 mm in thickness [13]. Kelly found histologic satellitosis in 6 of 216 (2.8%) of melanomas <3.0 mm in thickness, compared to 22 of 60 (37%) of melanomas >3.0 mm in thickness [14]. Kopf found histologic satellitosis in only 1 of 144 patients (0.7%) with melanoma <1.5 mm in thickness, compared to 29 of 102 patients (28%) with melanomas ≥1.5 mm in thickness. Leon found that 8% of patients with vertical growth phase melanoma have evidence of satellitosis [15]. Furthermore, he made the clinical observation that patients with microsatellitosis had an increased incidence of local failure, regional recurrence, as well as decreased disease-free and overall survival. Kelly echoed this observation, noting that, of patients with melanomas >3.0 mm in thickness, local recurrence was seen in 5 of 22 (14%) when satellitosis was present, compared to only 1 of 38 (3%) in the absence of satellitosis [14]. These observations began to set the stage for more conservative treatment of thinner primary melanomas.

A number of retrospective clinical observations have been made over the last decade to support more conservative management of thinner primary lesions. Urist found a local recurrence rate of 0.1% among 1051 patients with melanomas <1.0 mm thick, 62% of whom were treated with margins of <2.0 cm [16]. Cosimi found no local recurrences in patients with low-risk primary melanomas, Clark II-III, 0.3–1.2 mm in thickness following wide excision and primary closure, with margins ranging from 0.7 to 4.0 cm [17]. Bagley reported on 147 patients with melanoma seen at the Lahey Clinic, where an excision was considered adequate if a margin of two times the diameter of the primary lesion was achieved. Inadequate margins were associated with increased local recurrence in patients with intermediate or high-risk melanoma, but did not adversely impact on long-term survival when compared to those patients undergoing 'adequate' excisions [2]. Schmoekel reported on 577 patients with clinical Stage I melanoma. Margins

were found to correlate with local recurrence; a local recurrence rate of 2.0% was seen in patients with margins >3 cm, compared to 10% in patients with excision margins of <3 cm ($p = 0.02$) [18]. However, survival did not correlate with margins. They recommended a minimum margin of 3 cm of all melanomas to minimize local recurrence. Goldman reported on 45 patients, all with primary melanomas <0.85 mm in thickness, all treated with surgical margins of ≤2 cm. He noted no locoregional recurrences in this favorable group [19]. Kelly noted an 8% local recurrence in melanomas treated with a <1 cm margin, compared to 0.3% recurrence in patients treated with greater >1 cm margin [14]. This recurrence rate was not stratified by primary melanoma thickness.

The most important retrospective observation was made by Cascinelli, reviewing local recurrence rates in 593 patients with clinical Stage I melanoma [20]. In patients with melanomas ≤2.0 mm in thickness, those patients undergoing narrow excision with <2 cm margins had a local recurrence rate of 5%, comparable to the 2% local recurrence rate of those patients undergoing wide excision with ≥3 cm margins. However, in patients with thicker melanoma, >2.0 mm, patients undergoing conservative excision with margins <2 cm, the local recurrence rate was 12%, significantly greater than when those same patients underwent wide excision, where the local recurrence rate was only 4%. Although the local recurrence rate was increased in patients with thicker melanomas undergoing conservative excision, he found no correlation between surgical margin and survival.

Based on these retrospective observations, the World Health Organization (WHO) embarked on a prospective randomized trial comparing 1 cm vs. 3 cm margins in patients with primary melanomas <2.0 mm thick. The results of this trial were initially reported in 1988, then updated in 1991 [21,22]. Three hundred five patients were randomized to receive a 1 cm margin, compared to 307 who had margins of ≥3 cm. In this trial, with a median follow-up of 90 months, there were only four local recurrences, all of the narrow excision group. All patients who recurred had melanomas >1.0 mm thick, all Clark Level IV. In the study as a whole, there was no statistical difference in the incidence of local recurrence, or of disease-free or overall survival, comparing the narrow to the wide excision groups. Results of this trial would suggest that, at a minimum, all patients with melanomas measuring <1.0 mm in thickness can be safely managed with 1 cm margins. As 4 of 113 patients (4%) with melanomas measuring 1–2.0 mm in thickness undergoing narrow excision recurred locally, it is not clear from this trial that this represents the safest margin for these patients.

The National Intergroup Melanoma Trial has recently completed accrual, randomizing patients with melanomas measuring 1.0–4.0 mm thick to 2 vs. 4 cm margins. While the results of this trial have not been published, a presentation of preliminary results has suggested no difference in local recurrence or survival in these groups [23].

From the above, it would appear that satisfactory margins for primary

Table 2. Primary cutaneous melanoma: recommended surgical margins

Thickness	Margin
In situ	0.2 cm
≤1.0 mm	1 cm
1.1–4.0 mm	2 cm
>4.0 mm	≥3 cm

melanomas measuring <1.0 mm thick would be 1 cm, while for those measuring 1–4.0 mm in thickness, a 2 cm margin would be adequate. For primary melanomas measuring >4.0 mm thick, the optimal surgical margin is unknown but should probably be at least 3 cm (Table 2).

There are a number of special sites where surgical margin should be tempered with clinical judgement. On the sole of the foot, every effort should be made to preserve some of the weight-bearing plantar skin. Most digital melanomas require amputation. While the amputation is usually performed at the metatarsal/metacarpal head, interphalangeal amputation may be indicated for subungual melanomas of the thumb to preserve adequate function. Melanomas of the face are often excised with more conservative margins for cosmetic reasons. Melanomas of the ear may be dealt with by wedge excision of the helix, reserving major auricular amputation for locally recurrent disease [24].

2.4. Local recurrence

Although local recurrence following adequate treatment of primary melanoma is unusual, it may appear as a surgical problem. Certainly, any recurrence within 5.0 cm of the primary site should be initially addressed as a local recurrence. Appropriate surgical management of these patients usually requires wide excision with split-thickness skin graft. The role of adjuvant hyperthermic isolation limb perfusion in patients undergoing excision of locally recurrent extremity melanoma is unclear. However, it appears that local recurrence is often a harbinger of future systemic disease. Long-term survival in patients with local recurrence is between 20% and 30% [16].

3. Surgery for regional lymph nodes

Although the majority of patients who are newly diagnosed with malignant melanoma present with localized disease [1], at least 8% present with regional nodal metastases, and perhaps another 10% will develop nodal metastases during the course of their disease. There is no other topic in the surgical management of malignant melanoma that has aroused so much controversy

as the management of the regional nodes in these patients. In particular, the role of elective lymphadenectomy in patients with clinically negative nodes continues to be a subject of debate, despite a number of prospective randomized trials, which will be discussed below. The extent of therapeutic lymphadenectomy in certain anatomic regions to both maximize survival as well as minimize morbidity is also controversial.

3.1. Prognostic factors

Patients with melanoma metastatic to regional nodes are prognostically quite heterogeneous, with reported 5-year survival rates ranging from 8% to 76% [25,26]. It is important to understand the factors that govern prognosis in these patients in order to fully appreciate the impact of regional lymph node dissection. A large number of factors have been examined, including patient age and sex, disease-free interval, primary tumor Clark level, thickness, ulceration and site, number and percentage of involved lymph nodes, and the presence of extranodal tumor extension. Of these single factors, those measures of tumor burden, particularly the number of positive nodes and the presence of extranodal tumor extension, seem to be the most powerful predictors of outcome. Several authors have incorporated factors found to be significant by multivariate analysis into equations that weigh the relative contribution of each factor to predict the risk of recurrence in an individual patient [27–29].

3.2. Technique of lymphadenectomy

The technique of lymphadenectomy is fairly standard and has been well described [30]. A superficial groin dissection involves removal of all of the fatty and lymphatic tissue overlying the femoral triangle. An incision is made over the femoral vessels to the apex of the femoral triangle. The frequent incidence of skin slough at the wound edges can be obviated by excision of a 3 cm wide ellipse of skin overlying the femoral vessels. There is invariably enough skin for primary closure; if not, skin, grafting can be used over a transposed sartorius muscle. Skin flaps are raised laterally to the sartorius muscle, medially to the adductor magnus muscle, and inferiorly to the point where these two muscles cross, at which point the greater saphenous vein is encountered, and divided. Four to 6 cm of fatty and lymphatic tissue are dissected off of the external oblique muscle. The lymph node group is then dissected off the femoral vessels, exposing the branches of the femoral nerve in the process.

The saphenofemoral junction is divided as the greater saphenous vein exits the lymphatic package. The nodes are dissected off the common femoral vessels, with the highest node dissected being Cloquet's node, the bridging node between the superficial inguinofemoral and deep iliac and obturator nodes, and lies in the femoral canal, immediately medial to the common

femoral vein. Closure of the superficial groin wound involves a transposition of the sartorius muscle, rotating it from its origin on the anterior superior iliac spine over to cover the femoral vessels. This not only protects the femoral vessels in the event of significant skin slough, but also helps to prevent any postoperative femoral hernia. Closed suction drains are routinely employed, left in place until drainage is less than 50 cc a day, or 3 weeks, whichever occurs first.

If a deep groin dissection is to be done at the same time as a superficial groin dissection, the incision is extended cephalad towards the anterior superior iliac crest. The retroperitoneal space is entered, and the peritoneum and its contents are swept medially, exposing the iliac fossa. The pelvic lymph node dissection is started at the aortoiliac bifurcation, sweeping all of the lymphatic tissue down towards the femoral canal. The lateral border of this dissection is the iliofemoral nerve. The medial border is the peritoneum. A complete common iliac, external iliac, internal iliac, and obturator lymphadenectomy is undertaken. The obturator nerve and vessels are exposed and routinely preserved in the course of this dissection; if tumor clearly involves these structures, they may be sacrificed with minimal functional morbidity. If deep groin dissection is temporally remote from the superficial groin dissection, the same exposure can be achieved with a skin crease iliac incision. Closure of the deep groin dissection is done by primary intention, and suction drains are not required.

For axillary dissection, a skin crease incision is preferred, raising skin flaps anteriorly to the pectoralis major muscle, inferiorly to the fourth intercostal space, posteriorly to the latissimus dorsi muscle, and superiorly to the axillary vein. For therapeutic axillary dissection, our preference is to include the pectoralis minor muscle as well as the intercostobrachial nerve and thoracodorsal neurovascular bundle in the dissection. The long thoracic nerve is preserved unless in proximity to gross tumor. For elective axillary dissection, some or all of these structures may be preserved. In either case, the dissection includes all three levels of the axillary nodes, Level III medial to the pectoralis minor muscle, Level II deep to the pectoralis minor muscle, and Level I lateral to the pectoralis minor muscle. The dissection is begun at the apex of the axilla, exposing the costoclavicular (Halsted's) ligament, then dissecting all of the fatty and lymphatic tissue off of the axillary vein. The dissection may be extended above the axillary vein if there is clinical nodal involvement in this region. The dissection includes a thorough removal of all nodes in the subscapular fossa. Primary closure over suction drains is routine, and wound complications tend to be minimal. Guidelines for removal of the drains are similar to those outlined above for the superficial groin.

A number of techniques for both radical and modified radical neck dissection for melanoma have been described. In essence, this involves raising skin flaps deep to the platysma muscle, inferiorly to the clavicle, superiorly to the angle of the mandible, medially to the midline, and posteriorly to the posterior cervical triangle. The cervical nodes are then dissected

out, preserving the thyroid, submandibular gland, and carotid artery. In a classical radical neck dissection, the sternocleidomastoid muscle, internal jugular vein, and spinal accessory nerve are all taken with the specimen. Innumerable modifications of this procedure have been described, both to limit the number of lymph node areas dissected to those in proximity to the primary tumor, as well as to preserve a number of the structures that are routinely sacrificed in the classical radical neck dissection. Primary closure over suction drains is routine, with guidelines for drain removal similar to that outlined above.

3.3. Morbidity

The long- and short-term morbidity of regional lymph node dissection for melanoma can be considerable. Ballantyne reported that 80% of 107 patients undergoing groin dissection required more than 21 days to complete wound healing [31]. Reported overall immediate wound-related complication rates range from 28% to 70% [32–36], and are summarized by site and type of complication in Table 3. The average length of hospital stay is prolonged by 7–12 days by a wound complication [32–34]; in one series wound complications were responsible for 17% of the total inpatient hospital days required by patients undergoing lymphadenectomy [32].

Coit looked at the impact of antibiotics in reducing the rate of immediate postoperative wound complications in a prospective randomized trial [32].

Table 3. Locoregional morbidity of regional lymphadenectomy

Author	Year	N	Necrosis (%)	Seroma (%)	Infection (%)	Edema (%)	LOS (Days)
Cervical							
Bland [34]	1981	133	5 (4)	22 (17)	5 (4)	2 (2)	12
Urist [33]	1983	48	5 (10)	5 (10)	2 (4)	0 (0)	8
Bowsher [134]	1986	15	NS	NS	1 (7)	1 (7)	0 (0)	NS
Shaw [36]	1990	99	4 (4)	10 (10)	9 (9)	4 (4)	12
Axillary							
Bland [34]	1981	87	9 (10)	23 (26)	9 (10)	NS	16
Urist [33]	1983	98	0 (0)	26 (27)	7 (7)	1 (1)	8
Bowsher [134]	1986	30	NS	NS	9 (30)	9 (30)	1 (3)	NS
Shaw [36]	1990	51	1 (2)	6 (12)	6 (12)	4 (8)	8
Karakousis [135]	1990	133	1 (0.8)	9 (7)	7 (5)	5 (4)	NS
Groin							
Bland [34]	1981	110	7 (6)	52 (47)	7 (6)	NS	22
Arbeit [35]	1981	44	7 (16)	7 (16)	3 (7)	2 (5)	15
Urist [33]	1983	58	1 (2)	13 (23)	3 (5)	14 (26)	9
Bowsher [134]	1986	46	NS	NS	16 (35)	14 (30)	16 (35)	NS
Shaw [36]	1990	52	10 (19)	12 (23)	25 (48)	23 (44)	NS
Baas [37]	1992	151	5 (3)	25 (16)	14 (9)	30 (20)	13

He found that the addition of perioperative cefazolin was associated with fewer wound complications following axillary, but not inguinal, dissection. He concluded that wound complications after groin dissection are due more to technical than microbiologic factors.

Baas and his group from Gronigen in the Netherlands, described a technique of excision of an 8 cm wide ellipse of skin over the femoral triangle to minimize skin edge necrosis [37]. Using this technique, they reported wound edge necrosis in only 5 of 151 patients (3%). In either case, it is quite clear that the rate of complications reported in prospective trials greatly exceeds the rate of complications recovered from chart review in retrospective trials. Radical lymphadenectomy is not a benign procedure with respect to morbidity, and this morbidity must be weighed against potential benefits in any discussion about elective lymph node dissection.

3.4. Elective lymphadenectomy

There is no topic in the surgical management of malignant melanoma that has aroused so much controversy as the topic of elective lymphadenectomy. Proponents point to the clear survival advantage demonstrated in numerous retrospective reviews when comparing the outcome of patients undergoing elective lymphadenectomy for clinically negative, but histologically positive, nodes when compared to those patients undergoing therapeutic lymphadenectomy for clinically and histologically involved lymph nodes. Opponents point to the results of a number of prospective randomized trials as showing the procedure to have no benefit.

Historically, elective lymphadenectomy for metastatic malignant melanoma was based on the observation that, with increasing depth of invasion or thickness of primary lesion, clinically negative regional lymph nodes harbored occult metastatic melanoma with increasing frequency [38–40]. In addition, it was felt that with melanoma of intermediate thickness, the risk of regional lymph node metastases exceeded that of systemic metastases [41]. In these patients, an aggressive locoregional approach to the disease was felt to be much more likely to impact on outcome. In very thin melanomas, the risk of either locoregional or systemic metastases was felt to be so low as to not warrant the morbidity of the procedure. In thicker melanomas, some series have suggested that the risk of distant metastatic disease is so great that aggressive locoregional management does not impact on outcome [42].

A number of retrospective series have been published to justify this observation. Balch, in a review of end results of 1786 patients with clinical Stage I melanoma from a combined University of Alabama and Sydney Melanoma Unit Registry, found a significant survival advantage when comparing patients with clinical Stage I melanoma initially managed with wide excision and regional node dissection to those initially managed with wide excision alone, when their primary tumors measured 1.5–3.99 mm in thickness [43]. Most, if not all, of that survival advantage was confined to patients who

were found to have occult positive nodes at elective lymphadenectomy. However, it is important to recall that this intermediate thickness group of patients represented only 27% of all patients in the series, accrued over a period of 25 years.

In a multivariate analysis of risk factors for patients with malignant melanoma, he was able to classify patients into low risk of either systemic or regional metastatic disease, intermediate risk, i.e., at risk for regional but not systemic disease, and those at high risk for systemic disease who would not benefit from a regional node dissection. This classification was made based not only on tumor thickness, but also the presence of ulceration. When looking at the group at intermediate risk, he once again confirmed the survival advantage of patients undergoing elective lymph node dissection as a part of their initial management. This observation has been confirmed by some [44,45] but not all [46,47] retrospective series published in the interim (Tables 4 and 5). In general, however, it appears from these reported series that overall survival outcome is better when patients with clinically occult metastatic disease to regional lymph nodes undergo regional lymph node dissection when compared to their counterparts with clinically evidence

Table 4. Survival after elective lymphadenectomy for melanoma metastatic to regional nodes

Author	Year	N	5 yr (%)	10 yr (%)	Comment
Callery [136]	1982	29	48	—	All sites
Veronesi [48]	1982	54	50	37	Distal extremity primaries
Roses [137]	1985	157	44	28	All sites
Koh [26]	1986	66	49	—	All sites
Karakousis [135]	1990	52	60	—	Axillary nodes
Morton [28]	1991	NS	59	51	All sites
Coit [29]	1991	99	52	43	Axillary and inguinal nodes

Table 5. Survival after therapeutic lymphadenectomy for melanoma metastatic to regional nodes

Author	Year	N	5 yr (%)	10 yr (%)	Comment
Chang [138]	1982	75	36	41	Unknown primary
Callery [136]	1982	119	36	—	All sites
Roses [137]	1985	56	20	12	All sites
Wong [139]	1987	188	42	40	Unknown primary
Jonk [140]	1990	26	49	49	Unknown primary
Karakousis [135]	1990	52	13	—	Axillary nodes
Karakousis [64]	1991	57	39	—	Inguinal nodes
Coit [29]	1991	254	35	31	Axillary and inguinal nodes
Morton [28]	1991	NS	43	38	All sites
Velez [141]	1991	34	45	35	Unknown primary
Spiro [58]	1992	63	22	22	Cervical nodes

nodal disease. This is not solely due to 'lead time bias,' that is to say, intercepting the nodal metastases earlier. If that were the case, although the median survival would be improved in the elective lymph node dissection group, the 5-year survival would be identical. However, it is important to recall again that this survival advantage is only seen in patients undergoing elective lymph node dissection who have positive lymph nodes, approximately 15–20% of all candidates for elective node dissection. It does not appear that elective lymph node dissection has any impact at all on the survival of patients in whom negative nodes are removed. Those patients suffer the morbidity of the procedure without discernible benefit.

Based on these observations, in the 1970s the World Health Organization embarked on a large multicenter prospective randomized trial of wide excision plus elective lymph node dissection vs. wide excision only in patients with intermediate thickness melanoma of their distal extremities. With minimum 8-year follow-up, the end results were reported by Veronesi and his colleagues [48]. In no subset of patients did the addition of elective lymphadenectomy improve the outcome. In fact, when Veronesi examined the outcome of patients undergoing elective lymph node dissection who were found to have microscopic positive nodes to those who subsequently required therapeutic lymph node dissection for clinically evident nodes, there was no statistically different survival. He concluded that in these patients elective lymph node dissection ought not to be considered standard therapy.

There have been a number of criticisms of this study [49]. The first is that neither thickness or ulceration, the two most important prognostic variables in Balch's series, were taken into account in the WHO study. Ulcerated lesions were disproportionately represented in the elective lymph node dissection group, thus perhaps adversely impacting on the overall outcome in this group. Veronesi made available his data to Balch for analysis. When Balch applied his criteria of intermediate risk to the end results, he was able to find a trend towards improved outcome in the intermediate risk group, undergoing elective lymph node dissection when compared to those undergoing wide excision alone [50]. It is important to realize, however, that the intermediate-risk group comprised only a small proportion of the overall group, and that, while there was a trend towards improved survival, this trend was not statistically significant.

Sim and colleagues from the Mayo Clinic performed a single-center, three-armed randomized trial looking at patients with clinical Stage I malignant melanoma, randomized to receive wide excision alone, wide excision followed by elective lymph node dissection delayed 1 month, or wide excision with immediate elective lymph node dissection [51]. With $4\frac{1}{2}$ years of follow-up, they found no difference in overall or disease-free survival in any of the three groups. This trial has been criticized for small numbers in each group, and the large proportion of very low risk melanoma patients included.

The WHO is currently involved in an ongoing trial to evaluate the role of

elective lymph node dissection in patients with proximal extremity or truncal melanomas. The results of this trial are not as yet available.

Finally, the National Intergroup Melanoma Trial led by Balch and colleagues has recently completed accrual, with end results awaiting further follow-up. In this trial, patients with melanomas measuring 1.0–4.0 mm in thickness anywhere on the body were randomized to immediate elective vs. delayed therapeutic, if necessary, lymph node dissection. In addition, patients with melanomas of trunk or proximal extremity were randomized to undergo 2 vs. 4 cm surgical margins (discussed above). The results of this trial are as yet not available, but are eagerly awaited, as they will undoubtedly clarify the management of these patients.

It is probable that even a large prospective randomized trial to evaluate the impact of elective lymphadenectomy on outcome will be unable to show a statistically significant improvement in survival. The reason for this is quite clear. The survival advantage is only accrued to the small proportion of patients with histologically positive nodes. This survival advantage is diluted out by the lack of impact on survival in the majority of patients who have negative lymph nodes. Future strategies should focus not on applying this elective lymphadenectomy to all patients at risk, but to try to identify those patients at risk who have micrometastatic disease in regional lymph nodes whose outcome will be improved by early lymphadenectomy.

Morton has developed a technique of intraoperative lymphatic mapping using isosulfan blue in an effort to do this [52]. The technique is based on the theory that every site in the skin has a designated first draining lymph node and that melanoma cells preferentially travel through this pathway first to reach that draining lymph node, the 'sentinel node,' prior to traveling onto other areas. The technique is elegant in its simplicity. A small amount of blue dye is injected intradermally at the site of the primary melanoma. The afferent lymphatic channel, stained blue, is then identified as it enters the regional lymph node bed and is traced to the first draining node. If that node is found on frozen section to contain micrometastatic disease, a formal lymphadenectomy is taken. If that lymph node is negative, it can safely be assumed that the remaining lymph nodes are negative and no further surgery is undertaken.

Morton has validated this technique in 223 patients. In only 2 of 194 (1%) lymphadenectomy specimens was there melanoma in the dissected nodal bed in the presence of a negative sentinel lymph node. Although there is a significant learning curve to this technique, it may well define the definitive plan of management in patients with intermediate-risk melanomas.

3.5. Therapeutic lymphadenectomy

In a patient with regional lymphadenopathy suspicious for metastatic melanoma, histologic confirmation is advisable prior to definitive lymphadenectomy. This can be achieved either by lymph node biopsy, or preferably,

fine needle aspiration cytology. Excision of the clinically suspicious lymph node alone is inadequate for therapy. Kane and associates found residual melanoma in 15 of 22 lymph node dissections (68%) following diagnostic excision of a clinically suspicious and histologically positive node [53].

Although there remains considerable debate as to whether or not melanoma adheres to the Halstedian principle of metastatic disease, with tumor cells metastasizing from the primary to regional nodes before systemic dissemination there is very little disagreement that resection of clinically and histologically involved regional nodes from metastatic melanoma results in a finite long-term disease-free survival (Table 5). Thus, in at least a proportion of patients, therapeutic lymphadenectomy encompasses all clinically relevant disease. The only real area of controversy with regard to therapeutic lymphadenectomy relates to the extent of the procedure to be performed.

In the head and neck region, most proponents of elective lymphadenectomy advocate a policy of selective, modified radical neck dissection for patients with clinically negative cervical nodes [54–58]. This policy spares these patients the long-term morbidity of a full radical neck dissection, while minimizing the probability of locoregional failure; as stated previously, the impact of elective cervical lymphadenectomy on survival remains controversial.

The role of modified radical neck dissection in the management of patients with clinically evident nodal metastases is less clear. Shah has reported the high frequency of involved nodes at multiple levels in patients undergoing therapeutic cervical lymph node dissection, implying that any procedure less than a complete radical neck dissection in this setting risks leaving residual disease behind [59]. Byers noted a 50% recurrence rate in the neck in patients undergoing modified radical neck dissection for clinically palpable disease [54]. Even after formal therapeutic radical neck dissection, nodal recurrence rates of 10–44% are reported, the rate depending on factors such as the number of involved nodes and the presence of extracapsular disease extension [55,60]. Although conclusive data regarding the extent of therapeutic neck dissection necessary to minimize nodal recurrence and maximize survival are unavailable, most authors feel that a complete radical neck dissection is warranted in these patients.

The role of postoperative adjuvant radiation therapy for patients with resected melanoma metastatic to cervical nodes to diminish the high rate of regional failure also remains controversial. In a recent retrospective report by Ang and colleagues on 48 patients with palpable cervical nodes treated with post lymphadenectomy radiotherapy, the locoregional failure rate was 10% [26], an incidence less than expected based on historical series. The efficacy of this modality is currently being evaluated in a multicenter randomized trial.

Controversy also exists as to the role of parotidectomy in patients with melanoma metastatic to nodes in that region. Most authors feel that elective superficial parotidectomy is warranted in those patients with periauricular or scalp primary tumors, where there is a substantial risk of intraparotid lymph node involvement [61,62].

Table 6. Survival of patients with melanoma metastatic to deep pelvic nodes

Author	Year	N	No. 5-yr survivors (%)
Fortner [142]	1964	46	4 (9)
McCarthy [75]	1974	4	0 (0)
Cohen [143]	1975	3	0 (0)
Finck [144]	1982	23	4 (12)
Jonk [145]	1988	23	3[a] (13)
Coit [63]	1988	33	1 (3)
Karakousis [64]	1991	28	6 (21)
Singletary [146]	1992	8	0 (0)
Total		168	18 (11)

[a] All with unknown primary site.

A second area of controversy regarding the extent of therapeutic lymphadenectomy focuses on the indications for deep pelvic lymph node dissection in patients with melanoma metastatic to superficial inguinal nodes. Proponents of routine iliac lymphadenectomy in those patients cite the probability of deep iliac nodal involvement in the presence of clinically involved superficial groin nodes as being 30–40% [63,64]. Opponents of routine pelvic node dissection site the lack of therapeutic advantage to removing additional histologically negative nodes and the very high probability of death from systemic melanoma in patients with any histologic involvement of pelvic nodes (Table 6).

In a collected series of over 400 reported patients undergoing combined inguinofemoral and deep pelvic lymphadenectomy for melanoma (Table 7), positive superficial inguinal nodes were associated with positive pelvic nodes in only 17% of patients. Patients with clinically positive inguinofemoral nodes were found to have deep pelvic nodes 36% of the time (Table 8). Coit

Table 7. Probability of positive deep pelvic nodes with positive superficial groin nodes

			Deep nodes	
Author	Year	N[a]	Negative (%)	Positive (%)
McCarthy [75]	1974	12	9 (75)	3 (25)
Cohen [143]	1975	23	20 (87)	3 (13)
Finck [144]	1982	82	58 (71)	24 (29)
Coit [63]	1988	90	71 (79)	19 (21)
Illig [147]	1988	75	62 (83)	13 (17)
Singletary [146]	1992	131	123 (94)	8 (6)
Total		413	343 (83)	70 (17)

[a] N = number of patients with positive superficial groin nodes.

Table 8. Probability of positive deep pelvic nodes by clinical status of positive superficial nodes

Author	Year	Number of patients with positive deep nodes (%)	
		Superficial nodes clinically negative (%)	Superficial nodes clinically positive (%)
Cohen [143]	1975	0/5 (0)	3/18 (17)
Finck [144]	1981	5/16 (31)	20/25 (80)
Coit [63]	1989	3/47 (6)	29/103 (28)
Karakousis [64]	1991	6/31 (19)	21/57 (37)
Total		14/99 (14)	73/203 (36)

and associates found that the most accurate predictor of deep pelvic node positivity was involvement of Cloquet's lymph node, the lymph node in the femoral canal, immediately medial to the femoral vein, bridging the superficial and deep pelvic nodal groups. In his series, 19 of 24 patients (79%) with a positive Cloquet's node were found to have positive deep pelvic nodes. In the absence of any other curative modality, he advocated a policy of selective pelvic lymphadenectomy for patients with clinically or radiologically evident involvement of deep pelvic nodes, or for those patients with a very high likelihood of positive deep pelvic nodes (i.e., those with a positive Cloquet's node).

While involvement of the two contiguous regional node areas of the superficial and deep groin portends a very poor prognosis, less consistent results have been reported following dissection of more than one anatomically separate lymph node areas, such as bilateral axilla, or axilla and groin. While some authors have reported a very poor survival in this subgroup of patients [65,66], Barth and associates reported a 27% five-year survival among 21 patients with melanoma metastatic to at least two nodal basins [67]. It was their feeling that these patients ought not to be excluded from consideration for therapeutic lymphadenectomy with curative intent in the absence of distant metastatic disease.

3.6. Palliative lymphadenectomy

Regional lymph node dissection has a significant role in the management of patients with distant metastatic disease. Uncontrolled growth of regional lymph nodes in the neck, axilla, or groin can result in substantial locoregional morbidity. Regional lymph node dissection in these patients should be considered to limit that morbidity in the context of the estimated life expectancy of the patient. Locoregional control following lymphadenectomy is excellent, with recurrence rates of 0–33% reported (Table 9). The incidence of regional recurrence clearly increases with increasing tumor burden, as measured by the number of histologically positive nodes, as well as the

Table 9. Nodal basin recurrence after lymphadenectomy for melanoma metastatic to regional nodes

Author	Year	N	% with nodal recurrence	Comment
Santini [148]	1985	96	25	Cervical nodes, unknown primary
Singletary [60]	1986	287	15	Cervical nodes
Coit [63]	1989	203	8	Groin nodes
Belli [149]	1989	93	10	Cervical nodes
Calabro [122]	1989	287	15	Cervical nodes
		438	15	Axillary nodes
		276	17	Inguinal nodes
Bowsher [134]	1990	15	33	Cervical nodes
		30	13	Axillary nodes
		46	9	Inguinal nodes
Shaw [36]	1990	15	27	Cervical nodes
		34	29	Axillary nodes
		52	28	Inguinal nodes
Singletary [146]	1992	264	15	Inguinal nodes
O'Brien [55]	1992	150	28	Cervical nodes
Gadd [100]	1992	225	13	Axillary nodes
		224	9	Inguinal nodes
Baas [37]	1992	151	0	Inguinal nodes

presence of extracapsular disease [60,68]. Regional recurrence, once present; can present an extraordinary surgical challenge, and every effort should be made to avoid this, even in the patient with disseminated disease.

4. Surgery for regional disease

It is estimated that, of patients with malignant melanoma who relapse, 10–20% will recur with metastatic intransit melanoma, clinically confined to the arm or leg. From a biologic standpoint, the best evidence that the disease is in fact pathologically confined to the extremity in at least a proportion of these patients comes from a review of major amputations for advanced malignant melanoma by Jaques and colleagues [69]. In this review, curative major amputations (hemipelvectomy, hip disarticulation, or forequarter amputation) resulted in 42% five-year disease-free survival in 24 patients with multiple intransit metastases only. While not advocating amputation as the treatment of choice for these patients, the authors did make the point that the disease had been pathologically confined to the amputated extremity in those long-term survivors.

There are a number of options in treating patients with metastatic intransit melanoma, ranging from local excision through immunotherapy, isolation limb perfusion, and amputation. Each will be discussed separately below.

4.1. Natural history

In order to understand the impact of treatment on regional recurrence, it is important to appreciate the natural history of patients who present with this form of recurrence and factors that impact on prognosis. Wong, in a report of 95 patients with metastatic intransit melanoma, reported a 5-year survival of 12%, with a median survival of 19 months [70]. Features predictive of improved survival in his series were a disease-free interval of greater than 36 months and less than 10 intransit lesions at recurrence. Patients undergoing surgical treatment (local excision and/or HILP) had an improved survival over those patients undergoing nonsurgical treatment only. Singletary reported on 135 patients with metastatic intransit melanoma [71]. Factors predictive of improved survival in her series were the presence of intradermal or mixed (as opposed to purely subcutaneous) metastases, female sex, and surgical resectability, with or without HILP.

4.2. Local treatment

Local excision of metastatic intransit melanoma is occasionally indicated, particularly in a patient who recurs with a single recurrent intransit metastasis. Excision is also appropriate for both those patients who are not good candidates for either immunotherapy or limb perfusion, as well as for those patients with large painful, ulcerated or bleeding intransit metastases, lesions with little or no chance of meaningful response to any systemic, regional, or local injection therapy. The degree of palliation obtained by such procedures, even when they require skin grafting, can be substantial and should not be overlooked. While excision of all visible lesions can render a patient disease free for a period of time, the likelihood of further regional recurrence is high. In Singletary's series, 9 of 16 patients (56%) undergoing excision alone recurred regionally as their first failure site [71], while Hafston noted local or regional recurrence in 19 of 36 patients (53%) after surgery alone [72].

Waters and associates has recently reviewed their experience with carbon dioxide laser ablation of cutaneous metastases from malignant melanomas [73]. At a median follow-up of 8 months, fewer than 1% of over 2000 metastases treated had recurred locally. Of 30 patients treated in this manner, 16 (53%) recurred at other sites, requiring further treatment. This technique was felt to be appropriate for lesions up to 2 cm in diameter.

4.3. Immunotherapy

Morton and associates advocate a policy of initial management by intralesional immunotherapy with BCG for patients who recur with metastatic intransit melanoma [70]. Of 95 patients with metastatic intransit melanoma, they reported treating 42 with either intralesional BCG alone or in combination with systemic chemotherapy. They reported regression of over 90% of

lesions injected. These authors reserved hyperthermic isolation limb perfusion, in general, to patients who failed intralesional BCG.

4.4. Regional hyperthermic isolation limb perfusion

Creech and Krementz first described the technique of isolation limb perfusion in 1958 [74]. Cavaliere reported on the synergism between heat and high-dose chemotherapy in vitro in 1967 [75]. Stehlin confirmed these observations in humans in 1969 [76].

The technique of hyperthermic isolation limb perfusion has evolved over the last 30 years and is now quite standard. The vessels of the extremity are isolated; in the case of the upper extremity, this may be done via either an infraclavicular or axillary approach, while in the groin the vessels may be cannulated either above or below the inguinal ligament. Proximal and distal control is achieved, and the patient then systemically anticoagulated with heparin. The vessels are cannulated and the cannulae are then connected to a previously primed extracorporeal membrane oxygenator pump. All air is excluded from the pump circuit, and a tourniquet is applied to the limb, which both defines the level of cutaneous perfusion and minimizes any systemic leak of drug via subcutaneous collaterals. Extracorporeal circulation of the limb is then established, and the limb is warmed, both by the hyperthermic perfusate and by external heating blankets. Once satisfactory isolation and flow have been established, cytostatics are added to the perfusion circuit and are circulated for a specified period of time. Following completion of the limb perfusion, the limb is washed out with low molecular weight dextran, with the venous effluent discarded. The cannulae are removed and native circulation is restored.

Despite a large number of reports of limb perfusion for melanoma in the interim, it is exceedingly difficult to draw any firm conclusions as to the impact of this treatment modality in the management of patients with melanoma. Most reported series have been collected over a prolonged period of time, during which many different perfusion regiments are lumped together, treating patients with varying stages of disease in association with varying amounts of surgical resection performed concurrently with the perfusion. Many different perfusion protocols have been described, varying such factors as the perfusate, the degree of hyperthermia, the duration of perfusion, and finally, the drug used. Series have been reported using phenylalanine mustard, nitrogen mustard, DTIC, actinomycin-D, cisplatinum, as well as biologic agents, including monoclonal antibodies, interferon, and tumor necrosis factor [77].

For the purposes of the discussion to follow, limb perfusion can be divided into two broad categories, those that are prophylactic in intent, for patients with no measurable disease but at high risk for regional recurrence, and those of therapeutic intent, for patients with measurable intransit metastases.

4.4.1. Prophylactic limb perfusion. A large number of studies have been published over the last 20 years addressing the issue of the role of adjuvant isolation limb perfusion for patients with extremity melanomas at risk for locoregional recurrence. Taken as a whole, the conclusions in these series are difficult to interpret. Many patients treated with limb perfusion are compared to either historical or concurrent nonrandomized controls, and the conclusions are conflicting.

In 1975, McBride and associates reported on a series of 92 patients with localized melanoma of the extremity, greater than or equal to Clark Level III, treated with wide excision and adjuvant limb perfusion with melphalan [78]. Comparing these patients to a group of 72 historical control patients treated with wide excision and regional lymph node dissection only, they found a significant overall and disease-free survival advantage at both 5 and 10 years in the perfusion group.

In 1979, Golomb reported on 53 patients with invasive primary melanoma of the distal extremity treated with wide excision plus adjuvant HILP with melphalan, plus or minus regional lymph node dissection [79]. In comparing these results to 225 concurrent nonrandomized patients treated in a similar surgical fashion, although without limb perfusion, they found a trend toward improved overall and disease-free survival in patients with positive lymph nodes only. This difference in survival was not statistically significant.

In 1983, Rege reported a similar experience, looking at 39 patients with clinical Stage I melanoma of the distal extremity treated by wide excision, regional node dissection, and adjuvant HILP with melphalan [80]. In comparing these patients to 72 concurrent nonrandomized control patients undergoing wide excision and regional lymph node dissection only, they found an improvement in survival in the perfusion group, particularly for patients with lesions measuring >1.5 mm in thickness and for those less than 50 years of age.

In 1986, Fletcher reported on 23 patients with acral lentiginous melanoma treated with wide excision, HILP with melphalan, with or without regional node dissection [81]. In comparing their survival results to previously reported figures from the historical literature, they concluded that a marked improvement in survival could be achieved using adjuvant limb perfusion.

The drawbacks of using retrospective studies with nonrandomized control groups to assess the impact of therapy on outcome are exemplified by two consecutive reports from the same investigators in the Netherlands. In 1986, Martijn reported on a retrospective analysis of 120 patients with localized distal extremity melanoma, all Clark Level IV or V, and all greater than 1.5 mm in thickness [82]. These patients all underwent wide excision and adjuvant HILP with melphalan. They compared survival and recurrence patterns to a group of 116 computer-matched control patients from the Sydney (Australia) Melanoma Unit, with similar primary tumors treated by wide excision alone. As there were too few male patients and too few upper extremity lesions for comparison, their analysis was confined to females with

lower extremity tumors, 75 from the Netherlands and 67 from Sydney. They found a significantly improved overall, disease-free, and limb disease-free survival in patients undergoing perfusion. They concluded that for these patients adjuvant limb perfusion not only decreased locoregional recurrence, but improved 5- and 10-year survival rates, and that this should be considered standard therapy for these patients.

In 1988, however, Franklin [83], from the same institution, reviewed a 20-year experience evaluating the outcome in 227 patients with extremity melanomas >1.5 mm thick, undergoing wide excision and adjuvant HILP using melphalan, with or without concomitant elective regional node dissection. They compared their recurrence pattern and survival to a group of 238 matched control patients gathered from five regional hospitals undergoing wide excision of their primary tumor alone without adjuvant HILP. They found no statistically significant effect of perfusion with respect to time to recurrence in the limb, time to regional node metastasis, time to distant metastasis, or disease-free or overall survival. The discrepancy in the conclusions of these two series points to either a different biology for lower extremity melanoma in Australian woman relative to their counterparts in the Netherlands, or to the fallibility of using computer-matched controls to draw conclusions regarding the impact of treatment on outcome.

In 1989, Ghussen updated the end results of a small, but very important, prospective randomized trial to assess the value of adjuvant HILP in patients with localized melanoma of the distal extremity [84,85]. All patients had tumors >1.5 mm thick. Thirty-seven patients underwent wide excision, elective regional node dissection, and adjuvant HILP with melphalan, while a similar number of patients underwent similar surgical treatment without perfusion. At a median follow-up of 71 months, there was a statistically significant improvement in both disease-free and overall survival in the patients undergoing adjuvant HILP. Based on these results, they felt that adjuvant limb perfusion should be considered standard therapy for this group of patients.

In 1990, Edwards and colleagues reported on the outcome of 151 patients with extremity melanoma 'judged to be at high risk for local or regional metastatic disease,' treated with wide excision and adjuvant HILP, with either melphalan or DTIC [86]. These patients were compared to a similar number of computer-matched control patients derived from the University of Alabama and Sydney (Australia) Melanoma Unit databases, all treated with wide excision, without adjuvant HILP. With a median follow-up of nearly 4 years, they found no difference in disease-free or overall survival when comparing the groups as a whole. Subset analysis did show a slight improvement in disease-free and overall survival in patients with primary melanomas >2.0 mm thick perfused with melphalan, but this group comprised only 25 of the perfusion patients. They concluded that the results of this study did not support the routine use of adjuvant HILP as standard therapy in patients with localized extremity melanoma.

In 1991, Hafstrom and colleagues from the Swedish Melanoma group reported on the results of a prospective trial of 69 patients with recurrent melanoma of the extremity, randomized to treatment by either wide excision alone or wide excision with adjuvant hyperthermic limb perfusion using melphalan [72]. With a median follow-up of 36 months, there was a significant improvement in disease-free survival, as was a trend towards improved overall survival and fewer limb recurrences in the perfusion group.

In conclusion, data from retrospective trials are conflicting, in large part due to the inadequacy of retrospective trials to do anything other than define the natural history of a disease. The most provocative data come from Ghussen and Hafstrom, where in selected patients adjuvant limb perfusion did seem to impact both on recurrence and survival. These data clearly require confirmation. Two large multicenter prospective randomized trials are currently underway to evaluate the impact of adjuvant HILP with melphalan in patients with extremity melanomas measuring >1.5 mm in thickness. The results of these trials are eagerly awaited.

4.4.2. Therapeutic limb perfusion. Therapeutic hyperthermic isolation limb perfusion is indicated as the treatment of choice in patients with unresectable locoregional recurrence without evidence of systemic disease. Unfortunately, although objective response rates of 45–80% have been claimed (Table 10), the role of limb perfusion in improving or prolonging survival has yet to be shown in patients with metastatic intransit melanoma.

In 1981, Oldhoff and Koops reported on their experience with 100 patients with locally advanced malignant melanoma of the limbs, treated by wide

Table 10. Objective response after therapeutic isolation limb perfusion

Author	Year	Drug	Number	%
Hyperthermic perfusion				
Stehlin [76]	1969	l-PAM	10/12	83
Hafstrom [150]	1980	l-PAM	8/10	80
Storm [88]	1985	l-PAM	21/26	81
Shiu [90]	1986	H2-N	14/29	45
Skene [91]	1990	l-PAM	52/67	78
Coit [92]	1991	CDDP	10/15	67
Lienard [94]	1992	TNF/l-PAM	29/29	100
Normothermic perfusion				
Ryan [151]	1962	l-PAM	25/83	30
Stehlin [152]	1963	l-PAM	6/11	55
Bulman [153]	1980	l-PAM	14/29	48
Rosin [154]	1980	l-PAM	50/80	62
Kroon [155]	1987	l-PAM	15/18	83

l-PAM = melphalan or l-phenylalanine mustard; CDDP = cisplatin; H2-N = nitrogen mustard; TNF = tumor necrosis factor.

excision of all disease followed by HILP with melphalan with or without actinomycin-D [87]. Despite the fact that all gross disease was excised in these patients, the authors noted a 33% incidence of further recurrence in the limb following perfusion.

In 1985, Storm reported on a 10-year experience with 26 patients undergoing therapeutic HILP with melphalan for recurrent melanoma in the lower extremity; no effort was made to excise all gross disease [88]. The authors reported complete resolution of all tumor in 21 of 26 patients (81%). Sixteen of the 21 responses (75%) were durable, lasting over 4 years or until death. Of interest, however, there was no difference in overall survival when comparing the 16 patients with a sustained complete response in the limb, the five patients who had a transient complete response in the limb, or the five patients who had no response whatsoever. Only three patients of the entire group of 26 (12%) survived 5 years free of disease.

Sutherland had a less pessimistic view of patients undergoing hyperthermic isolation limb perfusion for metastatic intransit melanoma. Of 75 patients with M.D. Anderson Stage IIIA disease (intransit disease alone), he showed a median survival of 30 months, with a 5-year survival of 35% from the time of first perfusion [89].

In 1986, Shiu reported on the results of 42 patients undergoing therapeutic HILP with nitrogen mustard for metastatic intransit melanoma [90]. Of 29 patients with disease left in situ, an objective response was observed in 14 (45%). Eight patients had a complete response (27%), of whom only four were durable at 16–40 months postperfusion.

Ghussen, as a subset of the prospective randomized trial mentioned previously, reported on 16 patients with M.D. Anderson Stage III disease undergoing HILP after excision of all measurable metastatic intransit and/or nodal disease, comparing them to a similar cohort of patients undergoing surgical excision alone without HILP [84,85]. He found a significant improvement in both disease-free and overall survival in the perfusion group.

Finally, Skene in 1990 reported on 67 patients with measurable metastatic intransit disease undergoing HILP with melphalan; he observed a 78% objective response rate, although the proportion of complete responses was not reported [91]. Based on this observation, they felt that HILP was an active treatment modality, indicated for locoregional control and possibly limb salvage in these patients. No statement was made as to the impact of this treatment on survival.

A number of investigators have looked at other drugs, including cisplatinum (CDDP). Coit observed a 67% objective response rates in patients undergoing therapeutic HILP with CDDP. Forty percent of patients had a complete response, but only 20% of patients had a durable complete response [92]. Thompson, in a recent review, feels that CDDP is more toxic and no more effective than melphalan, and does not recommend its routine use for limb perfusion [93].

Most recently, Lienard and colleagues have reported on their experience

using tumor necrosis factor, alpha interferon, and melphalan in a HILP circuit for patients with metastatic intransit melanoma [94,95]. Although the potential morbidity and mortality of this treatment is high, they have reported an astounding 92% complete response rate. Oldhoff and Koops have confirmed both the response rate and toxicity of this regimen. To date, there have been no series in the United States evaluating this regimen.

In summary, the role of adjuvant HILP has yet to be proven and is the subject of ongoing prospective randomized trials. The role of therapeutic HILP in locoregional disease control has been firmly established, although its role in improving the survival of these patients is as yet unknown. If we can assume that 30% of all complete responders will be cured, it is quite clear that the durable complete response rate will have to be much higher than reported to date before an impact on survival is to be seen. The data from Lienard are most intriguing and require confirmation.

5. Surgery for metastatic disease

Hematogenous dissemination of metastatic melanoma often portends a very poor prognosis, with an overall median survival of less than 6 months and a 5-year survival of less than 5%. This group of patients, however, is quite heterogeneous, and a number of significant factors predictive of outcome have been identified. These would include involvement of skin and subcutaneous tissue as opposed to visceral disease [96–102], the number of metastatic lesions present [96,97,100], and the disease-free interval between the management of the primary tumor and the appearance of distant metastatic disease [96–99,103].

Furthermore, a number of authors have commented on the improved prognosis in patients whose metastatic disease is amenable to surgical resection [97,99,100,102]. In part, this reflects the improved performance status and lower tumor burden in these patients; however, it is important to note that in most series reporting on the outcome of patients with systemic metastatic melanoma, the only long-term survivors are those patients whose metastatic melanoma has been completely resected [99]. Thus, it would appear that in a small subgroup of carefully selected patients surgical resection may impact on the natural history of the disease, both by improvement in median survival, as well as the opportunity, albeit small, for long-term survival.

Apart from resection with curative intent, surgery plays another important role in the management of patients with hematogenous metastatic melanoma. Often these lesions are quite symptomatic, particularly those in the central nervous system or gastrointestinal tract. As will be shown below, excellent palliation can be obtained using surgical intervention in carefully selected patients. Surgery with palliative intent must be applied judiciously, however. Overett and Shiu, reporting on a series of 143 patients undergoing surgical resection for distant metastatic melanoma, found that patients undergoing

incomplete resection frequently spent prolonged periods of time in hospital, both from surgical complications as well as progressive disease, with many patients spending more than half of their remaining survival time in hospital [104].

As the intent of the surgical procedure, indications, and outcome are so different, we will next consider surgical intervention for distant metastatic melanoma by site of metastasis.

5.1. Nonvisceral metastases

Nonvisceral metastases predominate as the most common initial site of recurrence in those patients who relapse with distant metastatic melanoma [96,100,105]. This group of patients presents a unique opportunity for surgical intervention, with the individualized intent of either cure or palliation. Often these lesions can be ulcerating and painful, and excellent long-term local control with a minimum of morbidity can be achieved by local excision. More importantly, however, following complete resection this group of patients has a much better outcome than those with visceral metastatic disease. Following complete resection, the median survival in this group ranges between 17 and 50 months, with 5-year survivals between 9% and 35% (Table 11). Thus, aggressive surgical intervention is indicated for resectable remote skin, soft tissue, or nodal recurrence.

5.2. Lung

The lung is the visceral organ most frequently involved with metastatic melanoma during life, occurring in 12–18% of patients with an antecedent diagnosis of melanoma [98,106,107]. At autopsy, the lung is involved in 71–78% of patients who die of disseminated disease [108,109]. The usual

Table 11. Survival following resection of nonvisceral distant metastatic melanoma

Author	Year	N	Median (mo)	5 Year (%)	Comment
Amer [98]	1979	24	14	—	All patients
Feun [105]	1982	64	23	23	All resected patients, most with BCG
Balch [96]	1983		8	10	All patients
Overett [104]	1985	30	8.5	23	Remote nodes, resected
		16	20	9	Remote soft tissue, resected
Wornom [156]	1986	13	17	14	All resected, curative and palliative
Hena [157]	1987	44	17	16	All resected, curative and palliative
		32	29	—	Curative resection only
Markowitz [102]	1991	72	24	45	Remote nodes, resected
		60	50	61	Remote soft tissue, resected
Gadd [100]	1992	199	20	11	Single site, completely resected

presentation of pulmonary metastases is that of an asymptomatic nodule seen on screening chest x-ray [99,106,107]. This observation is important, as symptomatic patients derive very little benefit and have a very poor survival following surgical intervention for lung metastases.

It is important to be aware that not all solitary pulmonary nodules seen in patients with a prior history of melanoma represent metastatic disease. Up to one third of melanoma patients with a new solitary pulmonary nodule will be found to have either benign disease or a new primary [110,111].

Of patients with metastatic melanoma to the lung, apparently 12–25% are deemed operable [99,107], and of those operated upon 65–96% are found to be completely resectable [100,106,107,112]. Survival following complete resection of melanoma metastatic to the lung is the best of any visceral metastatic site (Table 12). Median survivals of 8–20 months are reported, with 5-year survivals ranging from 10% to 25%. These survival figures are a direct reflection of the care with which patients are selected for pulmonary resection.

Factors predictive of improved survival in patients undergoing pulmonary resection for metastatic melanoma include complete resection, a prolonged disease-free interval, and solitary metastasis only [107,112,113]. Harpole and Gorenstein both found that the presence of positive lymph nodes adversely affected outcome [106,107]. Harpole found the addition of postoperative adjuvant chemotherapy to improve outcome [107]. Wong found that the only survivors following pulmonary resection for metastatic melanoma were those patients whose tumor doubling time was less than 45 days [112]. Gorenstein, the only other author to examine this variable, did not find tumor doubling time to be predictive of outcome [106].

Table 12. Survival after resection of melanoma metastatic to the lung

				Survival	
Author	Year	N	Resectability %	Median (mo)	5 yr (%)
Mathisen [110]	1979	22	55	11	0
Morrow [158]	1980	8	—	—	12
Dahlback [159]	1980	8	—	7	0
Feun [105]	1982	26	—	16	15
Mountain [160]	1984	60	—	13	12
Thayer [161]	1985	18	—	17	11
Overett [104]	1985	47	36	8	9
Wornom [156]	1986	17	83	9	21
Pogrebniak [111]	1988	33	65	13	8
Wong [112]	1988	47	81	19	25
Marincola [162]	1990	9	—	18	12
Karp [113]	1990	29	76	10	4.5
Gorenstein [106]	1991	59	96	18	25
Harpole [107]	1992	98	89	20	20

In summary, in managing a patient with a prior history of melanoma with a solitary pulmonary nodule, the object of surgical intervention should be curative, not palliative. As such, a comprehensive extent of disease evaluation should be undertaken, including a CT scan of the head, chest, abdomen, and pelvis, as well as a bone scan. If all of these tests are negative and the tumor remains solitary during the evaluation, surgical intervention is appropriate. Furthermore, tumor doubling time should be estimated. If the patient is otherwise an acceptable surgical candidate, thoracotomy appears warranted. At operation, frozen section examination of the pulmonary nodule is mandatory, as there is a finite incidence of other pulmonary neoplasms for which the surgical procedure might well be altered by intraoperative knowledge of the histology.

5.3. Brain

Autopsy evidence of central nervous system involvement by metastatic melanoma is found in 49–73% of patients who die of disseminated melanoma [108,109]. Clinical evidence of metastatic disease to the brain, however, occurs in 8–18% of patients with melanoma who recur systemically [114,115]. These lesions are most often symptomatic, invariably those of a space-occupying lesion, usually headache, neurologic deficit, or occasionally acute onset of coma from hemorrhage. The initial management of any patient suspect of symptomatic brain metastases is the institution of high-dose corticosteroid therapy to relieve the swelling around the metastasis that causes symptoms. After therapy is initiated, radiologic evaluation with either a contrast-enhanced CT or gadolinium-enhanced MRI of the brain is undertaken. As the majority of patients with symptomatic central nervous system metastases have either antecedent or simultaneous extracranial metastatic disease, a complete extent of disease evaluation is warranted in these patients prior to any decision regarding surgical intervention. Those patients who are found to have solitary brain metastases in relatively accessible areas, and whose median life expectancy based on extent of disease exceeds 6 months, are generally felt to be good candidates for surgical resection. Of note, only the minority of patients with CNS metastases are considered for surgical resection [99,116].

The primary intent of surgical resection for melanoma metastatic to the brain is palliative rather than curative, and the degree of palliation is generally quite good. The procedures can be performed with a minimum of morbidity and mortality (Table 13). However, it is important to bear in mind that in a number of series, although long-term survivors are unusual, the only long-term survivors with CNS metastases are among those undergoing complete surgical resection [100,117,118].

The role of postoperative adjuvant radiation therapy following complete surgical resection is a matter of ongoing debate. DeAngelis and Hagen have shown a reduced relapse rate in the brain following CNS radiation,

Table 13. Results after resection of melanoma metastatic to the brain

Author	Year	N	Palliated (%)	Morbidity (%)	Mortality (%)	Survival Median (mo)	Survival 1,2,5 yr (%)
Fell [163]	1980	42	88	—	5.4	5	21 — 5
Galicich [121]	1980	13	—	—	—	6	29 6 0
Feun [105]	1982	16	—	—	—	15	— — 0
Madajewicz [114]	1984	20	—	—	15	6.5	15 10 5
Wornom [156]	1986	17	77	22	11	8	— — 13
Mendez [115]	1988	12	—	—	—	5.5	— 25 16
Guazzo [118]	1989	31	84	17	—	10	48 26 —
Brega [164]	1990	13	88	22	0	10	16 — —
Hagen [119]	1990	35	—	—	—	7	35 — —
Ordesson [165]	1990	40	63	20	5	8	— 25 15

but identical long-term survival when comparing patients undergoing postoperative adjuvant cranial radiation to those undergoing surgery alone for metastatic melanoma [119,120]. These investigators noted a 17% incidence of radiation associated dementia when daily doses in excess of 300 Cgy were employed.

Median survival following complete resection of melanoma metastatic to the brain ranges from 6 to 10 months, with a 1-year survival of 16–38% (Table 13). As mentioned above, occasional long-term survivors have been reported. Factors predictive of improved survival following surgical treatment of metastatic melanoma to the brain includes a prolonged disease-free interval, complete resection, and the extent of extracranial disease [119,121].

In summary, melanoma metastatic to the brain should be surgically resected when there is a solitary symptomatic lesion that is accessible to removal without major neurologic sequelae. Excellent palliation can be expected, although the majority of patients die of extracranial systemic relapse. If these criteria cannot be fulfilled, palliative radiation or expectant observation with corticosteroid therapy alone would be appropriate.

5.4. Gastrointestinal tract

Approximately 60% of patients who die from disseminated malignant melanoma will have evidence of gastrointestinal tract involvement at autopsy [108,109]. Less than 5% of melanoma patients develop clinical signs and symptoms of gastrointestinal metastases during life. Gastrointestinal metastases are the initial site of clinical metastasis following lymph node dissection in 1–3% of patients [100,122]. Within the gastrointestinal tract, melanoma has a clear predilection to metastasize to the small bowel, in part

Table 14. Results after resection of melanoma metastatic to the gastrointestinal tract

Author	Year	N	Palliated (%)	Morbidity (%)	Mortality (%)	Survival Median (mo)	Survival 1,2,5 yr (%)
Goodman [166]	1981	13	'Most'	—	19	4.5	— — —
Feun [105]	1982	9	—	—	—	18	— — —
Reintgen [126]	1984	110	90	—	0	8.5	— 15 —
Overett [104]	1985	23	—	—	—	9	— 22 13
Jorge [128]	1985	15	93	0	0	9	— 0 —
Wornom [156]	1986	7	100	33	14	—	— — —
Klaase [127]	1990	23	83	22	9	7.5	— — 19
Khadra [125]	1990	56	79	14	4	9.5	27 9 2
Ihde [123]	1991	32	94	—	3	6.2	27 19 0
Caputy [124]	1991	41	81	32	5	9.6	44 — 9

secondary to its length relative to the remainder of the gastrointestinal tract [123–127]. Most patients with melanoma metastatic to the gastrointestinal tract have multiple lesions [123,125], and most have clinical evidence of extragastrointestinal metastatic disease at presentation [123,124,126].

These patients present primarily with symptoms of pain, less often a mass. The most common objective findings include evidence of partial bowel obstruction or chronic, but rarely frank, gastrointestinal blood loss. Perforation is distinctly unusual as a presenting sign.

The long-term prognosis of these patients is poor, in large part due to the extent of disease at presentation. Appropriate patient selection for surgery again is a critical factor in outcome. In Roses' series, only 11 of 29 patients (38%) with melanoma metastatic to the gastrointestinal tract were felt to be suitable candidates for surgical intervention [99].

The clear intent of surgical intervention in these patients is palliative, not curative. With careful patient selection, the majority of patients undergoing surgical resection for melanoma metastatic to the gastrointestinal tract are well palliated with limited surgical morbidity and mortality (Table 14). Only occasional long-term survivors are reported, with the outcome generally governed by the presence of other systemic disease. Median survivals of 7–9 months are seen following surgical resection, with only very rare long-term survivors.

Factors predictive of improved survival include complete resectability [104,127], a prolonged disease-free interval [124], and the absence of other clinically evidence visceral metastatic disease [125,128]. Ihde noted a trend towards improved survival when only a single focus of metastatic melanoma was found in the gastrointestinal tract, but the numbers were so small that this did not achieve statistical significance [123]. Caputy noted the independently poor prognostic implications of small bowel involvement in

these patients [124]. He also noted a trend towards better survival with improving ECOG performance status.

In summary, the clinical diagnosis of melanoma metastatic to the gastrointestinal tract is unusual. Identifiable symptoms are well palliated by judicious surgical intervention, but long-term survival remains unusual.

5.5. Other sites

Melanoma can metastasize to virtually any site in the body. Ocular melanomas have a predilection for metastasizing to the liver, and these metastases are occasionally solitary [129]. Again, with careful patient selection occasional long-term survivors have been reported following resection of solitary hepatic metastases from melanoma. The majority of patients, however, have disseminated disease within the liver and are not candidates for surgical intervention. Mavligit has reported on hepatic infusion chemotherapy in these patients with limited success [130,131]. Melanoma metastasizes not infrequently to the breast. Survival following excision of breast metastases is comparable to that following resection of skin and soft tissue metastases, and should be routinely performed. Isolated reports of resection of melanoma metastatic to the adrenal [132], bone [104], and heart [133] have appeared, but again, long-term survival following resection at these sites is distinctly unusual.

6. Conclusions

In summary, a great deal has been learned about the biologic behavior of melanoma. This knowledge, derived from innumerable retrospective observations and tested in prospective trials, has defined the role of surgery in the management of patients with this disease. We are evolving a better understanding of how to manage primary melanomas based on criteria such as tumor thickness, to minimize local recurrence, and to maximize both survival and the cosmetic result. With regard to the management of regional nodes, we have not yet clearly defined that subgroup of patients, if indeed it exists, that might benefit from elective lymphadenectomy. Patients with clinically palpable melanoma metastatic to regional nodes present a spectrum of opportunity for surgical cure, a spectrum primarily defined by the extent of nodal disease. The appropriate procedure in the neck and groin necessary to maximize locoregional control and survival remains a matter of debate. While the role of adjuvant isolation limb perfusion remains unproven and investigational, therapeutic limb perfusion is clearly an active treatment modality in the management of patients with established intransit metastases. The optimal perfusion regimen has yet to be defined. Finally, surgical resection has an important role to play in the management of patients with distant metastatic melanoma. Careful definition of surgical intent, curative

or palliative, and rigorous patient selection and screening are mandatory to optimize end results in this challenging group.

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12. Chemotherapy of malignant melanoma

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1. Introduction

Malignant melanoma originates in its normal benign counterpart, the melanocyte, which is primarily located in the skin and eye. Occasionally, primary melanoma also occurs in the mucous membrane of the anus and external genitalia and, rarely, in visceral sites. The incidence of this disease is increasing rapidly on a worldwide basis, and in the United States it has doubled between 1980 and 1991. Melanoma has become the eighth most common malignancy in the United States (excluding nonmelanoma skin cancers), and in 1993 there will be approximately 35,000 cases and 6500 deaths. Twenty-five percent of cases occur in individuals 39 years or younger, and the highest incidence in the United States is found in Caucasians in southern Arizona, Hawaii, and across the 'sunbelt.'

At initial presentation, approximately 15% of patients have clinical evidence of disease spread beyond the local lesion. In about 85% of these patients, disease has spread no further than the regional nodes; in the others, there is evidence of distant metastases. Of those patients with only primary melanoma at the time of diagnosis, about one third develop metastases. Among the metastatic patients, although most initially experience metastases in soft tissue (lymph nodes, skin, or subcutaneous tissue), eventually metastases appear in other tissues and organs (liver, lung, brain, bone, or CNS). Five percent of melanomas are of unknown primary origin. Their natural history does not seem to differ from similarly staged metastatic melanoma of known primary origin. Forty-five percent of patients with melanoma that are not cured eventually develop clinically diagnosed central nervous system (CNS) metastases.

Ocular melanoma is the most common malignancy of the eye. Although the overall cure rate is at least as good as that of cutaneous melanoma, when metastatic 80% of patients experience liver involvement, with or without other sites of disease. Thus, the chemotherapy of metastatic melanoma of ocular primary site is often thought of as having a relatively poor result (see Chapter 7).

L. Nathanson (ed.), Current Research and Clinical Management of Melanoma.

1.1. Predictors of chemotherapy response

Overall melanoma has proved to be a remarkably chemoresistant tumor. When selecting patients with metastatic melanoma as candidates for chemotherapy, it is therefore important that favorable clinical factors predicting the likelihood of chemotherapy response be kept in mind. This is particularly true when designing studies of the efficacy of new therapeutic modalities. These favorable characteristics include:

1. Good performance status
2. Soft tissue disease or relatively small number of visceral sites (pulmonary metastasis appears to be the most sensitive)
3. Youth (<65 years of age)
4. No prior chemotherapy
5. Normal hemogram and normal hepatic and renal function
6. Absence of central nervous system metastases
7. Female gender

As in the systemic chemotherapy of all malignancies, the physician must remember that factors, including patient acceptance, adequate supportive care facilities, the economic and physical cost of the treatment, and the presence or absence of symptoms that may be palliated, must all be considered when selecting a patient for consideration for treatment with chemotherapy agents. Because none of our current regimens for therapy of melanoma are entirely satisfactory and true cures of metastatic melanoma with chemotherapy are uncommon, not all patients with melanoma are candidates for chemotherapy.

1.2. Staging

A brief summary of AJC TNM is shown in Table 1 [1]. At least four separate stages of patients would be eligible for chemotherapeutic intervention. Adjuvant studies could concern patients in (1) stage II (T3), (2) stage III (T4), or (3) stage-III (pathological N1, N2). On the other hand, chemotherapy of advanced and/or measurable disease could be considered in patients with (4) stage IV (M1A) or stage IV (M1B). Each one of these categories has somewhat different prognosis, and in reviewing the results of chemotherapy studies it is important to determine what the staging of the patients in such a study is and whether such stagings are clinical or pathological.

Clinical staging of primary melanoma consists of doing CBC, routine chemistries, chest X-ray, and any studies suggested by the patient's symptoms, physical examination, or laboratory findings. Use of routine CT scans, MRI, and GI films is done in patients who during the course of their disease have appropriate indications. Lymphangiography, lymphoscintography, radionuclide tomography, and PET scanning are all studies whose

Table 1. Staging cutaneous melanoma

Primary tumor (PT)	Regional lymph nodes (N)
pTX Primary tumor cannot be assessed	NX Regional lymph nodes cannot be assessed
pT0 No evidence of primary tumor	N0 No regional lymph node metastasis
pTis Melanoma in situ (atypical melanocytic hyperplasia, severe melanocytic dysplasia), not an invasive lesion (Clark's Level I)	N1 Metastasis 3 cm or less in greatest dimension in any regional lymph nodes(s)
pT1 Tumor 0.75 mm or less in thickness and invades the papillary dermis (Clark's Level II)	N2 Metastasis more than 3 cm in greatest dimension in any regional lymph nodes(s) and/or intransit metastasis
pT2 Tumor more than 0.75 mm but not more than 1.5 mm in thickness and/or invades to papillary-reticular dermal interface (Clark's Level III)	N2a Metastasis more than 3 cm in greatest dimension in any regional lymph node(s)
pT3 Tumor more than 1.5 mm but not more than 4 mm in thickness and/or invades the reticular dermis (Clark's Level IV)	N2b Intransit metastasis
pT3a Tumor more than 1.5 mm but not more than 3 mm in thickness	N2c Both (N2a and N2b)
pT3b Tumor more than 3 mm but not more than 4 mm in thickness	Distant metastasis (M)
pT4 Tumor more than 4 mm in thickness and/or invades the subcutaneous tissue (Clark's Level V) and/or satellite(s) within 2 cm of the primary tumor	MX Presence of distant metastasis cannot be assessed
pT4a Tumor more than 4 mm in thickness and/or invades the subcutaneous tissue	M0 No distant metastasis
pT4b Satellite(s) within 2 cm of the primary tumor	M1 Distant metastasis
	M1a Metastasis in skin or subcutaneous tissue in transit >2 cm from primary or lymph node(s) beyond the regional lymph nodes
	M1b Visceral metastasis

Stage grouping

Stage I	pT1	N0	M0
	pT2	N0	M0
Stage II	pT3	N0	M0
Stage III	pT4	N0	M0
	Any pT	N1, N2	M0
Stage IV	pT	Any N	M1

See: OH Beahrs, DO Henson, RVP Hutter, MH Myers (eds), American Joint Committee on Cancer 1988. Manual for Staging of Cancer, Philadelphia: JB Lippincott.

value for staging patients is still controversial. (See Chapter 6, where a similar non-AJC staging system is described).

1.3. Markers of melanoma progression

A major problem in rapid evaluation of melanoma has been lack of a biochemical marker for disease activity. Use of melanogen measurement in urine has not proven sufficiently sensitive for clinical use [2]. Recently the presence of the cellular adhesion molecule ICAM-I has been associated with metastatic potential in melanoma [3]. The detection of a circulating form of this molecule cICAM-I in blood has been correlated with the stage of melanoma [4]. The assay of essential growth factors such as bFGF could also provide markers for the future [5]. Radioimmunoimaging is also under study [6].

1.3.1. Organization of this review. The following review is organized with a goal of being 'user friendly' for the practicing oncologist. Thus the sections are divided into the number of agents to be used, currently commercially available vs. investigational agents (in the United States), and clinical setting (measurable metastatic disease vs. adjuvant chemotherapy). In addition, the three major useful agents in melanoma — DTIC (dacarbazine), BCNU, (carmustine), and cisplatin (platinol) — and their combinations have been the focus of the review. Other less active single agents are mentioned with appropriate references for those who wish to further investigate this field. Doses of principle drugs are mentioned in the text, but the reader should consult the bibliography for doses of other drugs. An emphasis is made on tabular summaries of data, rather than extensive narrative text. Bibliographic references emphasize recent literature and are listed primarily in the table for the convenience of the reader. A somewhat different format is used in multiple recent review articles on systemic therapy of melanoma, and they should also be perused by the interested reader [7–14].

2. Single-agent chemotherapy

2.1. Commercially available agents

The first widely used single chemotherapeutic agent thought to have specific antitumor activity in melanoma is dimethyltriazeno-imidazolecarboxamide (DTIC). In a recent review summarizing this data in 1868 patients from multiple investigators, 371 (20%) exhibited objective responses (Table 2) [15–21]. However, the most recently published data on the use of DTIC alone reported only 12% objective responses among 52 patients [17]. DTIC is an intravenous drug, usually used at a dose of 200 mg/m^2 IV on days 1–5 q 3 weeks, or 750 mg/m^2 IV on day 1 q 6 weeks. Toxicity includes mild

Table 2. Single-agent chemotherapy: single agents commercially available

Agents	Evaluable pts. CR + PR	Response	Response rate	Author	Year
DTIC	1868	371	20%	Mastrangelo	1992 [15]
				Pritchard	1980 [16]
DTIC	52	6	12%	Coccioni	1992 [17]
Nitrosoureas					
BCNU	122	22	18%		
CCNU	270	35	13%	Mastrangelo	1992 [15]
Methyl CCNU	347	54	16%		
Chlorozotocin	169	16	9%	Amburg	1982 [18]
Cisplatin	115	17	15.2%	Mastrangelo	1992 [15]
				Nathanson	1991 [19]
Carboplatin	43	17	16%	Casper	1990 [20]
Vinca alkaloids					
Vincristine	52	6	12%		
Vinblastine	62	8	13%	Mastrangelo	1992 [15]
Vindesine	114	17	15%		
Ifosfamide	60	7	12%	Varini	1987 [21]

fever, gastrointestinal toxicity (usually worse on the first day of treatment), as well as rare cases of hepatic venous occlusion (Budd-Chiari syndrome). The drug is relatively nonmyelosuppressive. The response rate in patients with soft tissue disease appears to be considerably higher than in patients with visceral metastatic disease.

All of the nitrosoureas have been used in malignant melanoma, with objective responses ranging around 18% for bis-chloroethyl-nitrosourea (BCNU, carmustine). This drug is given at approximately 150 mg/m^2 IV q 6 weeks and has a toxicity including gastrointestinal, marrow suppression (prolonged to 6–8 weeks), and occasionally pulmonary or renal toxicity. It is reconstituted in an alcohol-containing vehicle and may produce local burning and irritation at the site of injection. The other nitrosoureas, including cyclohexylchloroethylnitrosourea (lomustine), which is given in 100 mg to 130 mg/m^2 single dose PO q 6 weeks, have similar toxicities (other than those related to the route of administration). Although nitrosoureas are lipid-soluble drugs, therapeutic responses, or even prevention, of CNS metastases do not occur (see fotemustine below).

Cisdichlorodiaminoplatinum (cisplatin) has been shown to have a slightly over 15% response rate in 115 patients summarized recently. Standard doses of this drug are 100 mg/m^2 IV q 3 weeks. The drug has gastrointestinal, neurologic, auditory, and renal toxicity, and may produce hypocalcemia or rarely hemolytic-uremic syndrome. The chemical analogue of cisplatin, carboplatin, which is given at 400 mg/m^2 q 3 weeks, appears to have a similar response rate, although no prospective comparative study of the two drugs has yet been done. This latter drug is marrow suppressive, unlike cisplatin,

and may produce GI upset, but does not have the other toxicities of cisplatin. It has recently been suggested that cisplatin has a significant dose-response curve, and that doses above 120 mg/m^2 may be superior to those at standard doses [22]. When used at 150 mg/m^2, a response rate of 53% was reported by Glover [23] and of 35% by Avril [24] at 120 mg/m^2 (CDDP) using the chemoprotectant WR2721. Further studies will be needed to confirm this observation, which has been disputed by Buzaid [25]. Other drugs that have been observed as single agents to have activity in melanoma include the vinca alkyloids, cytosine arabinoside, procarbazine, dactinomycin, and ifosfamide (plus mesna). Of these, ifosfamide, used at a dose of 2000 mg/m^2 per day for five successive days IV, or 2400 mg/m^2 per day IV for three successive days, with hydration and mesna (given at approximately equal doses to the drug), appears to be the most promising of these agents.

2.2. *Investigational agents*

Table 3 [26–35] lists the most interesting new noncommercially available (USA) single agents that have been studied in melanoma in the recent past. Fotemustine is a nitrosourea that has been used widely in Europe with up to a 47% response rate. Complete remissions have been observed with this drug. Like other nitrosoureas, it has the potential of crossing the blood-brain barrier and may produce responses in patients with CNS metastases [27]. Doses are 100 mg/m^2 in 1 hour IV infusions weekly for 3 weeks, followed by a 4- to 5-week rest period, with response rates up to 24%. Toxicity is mainly hematologic. The drug has also been used in the intrahepatic arterial route of administration for hepatic metastasis, with a hepatic response rate of 61.5% [36].

Taxol is a new plant alkaloid that is used in 24 hour IV infusions at a dose of 250 mg/m^2 q 3 weeks. An overall response rate of approximately 14% has been observed, with complete responses reported. Primary toxicity

Table 3. Single-agent chemotherapy: single agents not commercially available

Agents	Evaluable pts.	Response (CR + PR)	Response rate	Author	Year
Fotemustine	19	9	47%	Schallreuter	1991 [26]
	153	37	24%	Jacquillat	1990 [27]
Taxol	28	4	14%	Einzig	1991 [28]
	25	3	12%	Legha	1990 [29]
Piritrexim	31	7	23%	Feun	1991 [30]
Dibromodulcitol				Simmonds	1985 [31]
Daily	120	24	20%	Bellet	1978 [32]
Intermittent	43	4	8.3%	Malden	1984 [33]
Temozolomide	23	4	17%	O'Reilly	1992 [34]
Detorubicin	22	8	36%	Chawla	1985 [35]

includes bone marrow suppression, and gastrointestinal tract and occasional anaphylatic reactions.

Piritrexim, a pyrimidine nonclassical antifolate, is given in 25 mg PO tid daily for 5 days for 3 weeks in each 4-week cycle. This schedule has produced a 23% response rate, primarily in soft tissue lesions. Dose-limiting toxicity is myelosupression.

Dibromodulcitol is an old drug with multiple clinical trials reported to produce up to 20% response rate in daily dosing. Temozolamide is an oral cytotoxic drug used at a dose of 150–200 mg/m^2 daily × 5. Gastrointestinal and marrow suppressive toxicities have been seen, with a response rate of approximately 17%.

Detorubicin, an anthracycline analog, showed a 36% response rate in a single study of 22 patients but has not yet been restudied to my knowledge. Its toxicities are similar to those of other anthracyclines.

3. Combination chemotherapy

Table 4A [37–40], Table 4B [41–49], and Table 4C [49–54] lists recent two-, three- and four-drug combination-chemotherapy, respectively, involving the use of at least 2 of the 3 major drugs (DTIC, nitrosoureas, and cisplatin). In order to compare the efficacy of these drugs, in addition to the objective response rates, approximate durations of remission are listed. Although few comparisons of single agents vs. multiple-agent chemotherapeutic regimens listed have been made in a prospective random fashion, it is the author's impression that combination chemotherapy yields a significant increase in the objective response rate and a modest increase in the median duration of response. However, it should be noted that complete remissions, responses of more than 24 months duration, and survivals of 2–5 years are uncommon with any of these regimens. One of the major problems with all of these therapies is the high incidence of relapse in the central nervous system or other sequestered sites, which tend to respond poorly to chemotherapy. Although the toxicity of the multidrug regimens is somewhat greater than that of single agents, deaths attributable to drug toxicity are uncommon.

A most disappointing aspect of combination chemotherapy has been the failure to observe a good dose-response relationship. It has been suggested by Murren [55] that a CDDP in total doses greater than 100 mg/m^2 (50 mg/m^2/day × 3) was associated with a higher response rate (50%) when used with DTIC. Luger [56], however, reported a 29% response using CDDP 40 mg/m^2/day × 5 and Steffens [57] a response rate of 17% (CDDP, 50 mg/m^2/day × 3) with DTIC. Furthermore, CDDP doses of 20 mg/m^2/day × 3 [58] with DTIC have produced a response rate of 24%, including complete responders. Data from marrow transplant studies [59] where very high doses of alkylating agents and nitrosoureas are used show higher partial

Table 4. Standard drug combination

Agents	Evaluable pts.	Response	Response rate	Median duration response	Author	Year
A. Two-drug combination						
DTIC + CCNU	55	5(CR) + 6(PR)	16%	3 months	Joensuu	1991 [38]
DTIC + methyl CCNU	122	18	14%	14 weeks	Costanza	1977 [37]
DTIC + BCNU	61	4(CR) + 8(PR)	20%	3–7 months	Costanza	1972 [39]
DTIC + CDDP	30	2CR + 9PR	37%	31 weeks	Fletcher	1988 [40]
B. Three-drug combination						
DTIC, vinblastine, CDDP	50	2CR + 18 PR	40%	9 m.	Legha	1990 [41]
DTIC, VCR, BCNU	40	3CR + 14PR	42.5%	4 m.	Cohen	1977 [42]
DTIC, dactinomycin chlorozotocin	30	1CR + 4PR	17%	31 wks	Samson	1982 [43]
DTIC, vindesine, CDDP	40	3CR + 12PR	38%	4 m.	Ringborg	1990 [44]
CDDP, BLEO, CCNU	25	OCR + 12PR	48%	4–82 wks	Cohen	1986 [45]
DTIC, CDDP, BCNU	20	2CR	10%	9–8 m.	McClay	1992 [46]
DTIC, CCDP, TAM	23	2CR + 1PR	13%	64 m.	Buzaid	1991 [47]
DTIC, HU, NU	95	9CR + 20PR	31%	27 wks	Costanzi	1975 [48]
DTIC, CDDP, procarbazine	13	2	15.4%	3.5 m.	Karakousis	1979 [49]
C. Four-drug combination						
DTIC, BCNU, CDDP, TAM	45	5CR + 18PR	51%	10–8 m. (med. surv.)	McClay	1992 [50]
DTIC, BCNU, CDDP, TAM	20	4CR + 7PR	55%	11 m.	DelPrete	1984 [51]
BLEO, eldesine, lomustine DTIC (BELD)	25	3CR + 6PR	45%	6.1 m.	Young	1985 [52]
BLEO, VCR, lomustine, DTIC (BOLD)	46	5CR + 5PR	20%	6.4 m.	York	1988 [53]
DTIC, CDDP, VCR, procarbazine	13	2PR	15.4%	3.5 m.	Karakousis	1979 [49]
DTIC, BCNU, VCR, chlorpromazine	121	12CR + 15PR	22%	9.9 m.	McKelvey	1977 [54]

response rates, but the proportion of complete responders and of sustained response (over 12 months) has been disappointing [60].

The combination that has been most actively studied in the recent past consists of cisplatin, 25 mg/m^2 IV on days 1–3 q 3 weeks, DTIC 220 mg/m^2 IV on days 1–3 q 3 weeks, carmustine 150 mg/m^2 IV once q 6 weeks, with and without Tamoxifen at 10–60 mg bid PO continuously. The combination with Tamoxifen was originally reported by Del Prete [51] to have a response rate of 55%.

The major comparative experience of the DTIC, BCNU, and cisplatin combination, with and without Tamoxifen, comes from the group of McClay et al. [46,50]. Their experience with successive groups of patients on study, with and without Tamoxifen, has suggested to them that Tamoxifen has a significant role in augmenting antitumor efficacy of this combination. The exact action of Tamoxifen in this setting is unclear. Studies with single-agent DTIC, plus or minus Tamoxifen [17], have suggested that Tamoxifen augments the efficacy of DTIC. On the other hand, in vitro data [46,50] implicate cisplatin as the possible target for Tamoxifen interaction. Contrary to in vitro data, Tamoxifen does not seem to augment the clinical efficacy of single-agent cisplatin. High-dose Tamoxifen at 160 mg/day × 7 days has been used and has been associated with a high proportion of CR but significant hematologic toxicity [61]. Random comparisons of the three-drug regimen, with and without Tamoxifen, are currently underway.

It should be noted that among the three-drug programs listed, DTIC, vinblastine and cisplatin and DTIC, BCNU and cisplatin appear to have a somewhat longer median duration of response.

A miscellaneous group of chemotherapy combinations are shown in Table 5 [17,21–82]. Some of these do not include DTIC, BCNU, or cisplatin. For example, procarbazine, 100 mg/m^2 q 1–5 PO, and dactinomycin, 1 mg/m^2 IV day 1, given q 3–4 weeks, can yield an approximately 25% objective response rate in individuals who have already failed cisplatin-, DTIC-, and BCNU-containing combinations. Whether carboplatin can be substituted for cisplatin in the combinations listed in Tables 4 and 5, avoiding nephrotoxicity and neurotoxicity, while preserving antitumor activity, has yet to be determined.

The addition of alpha-interferon to chemotherapy such as DTIC [83] or cisplatin [84] has been suggested to increase response rates in some studies but not in others [85]. Alpha-interferon will be discussed in Chapter 9 in more detail.

4. Adjuvant chemotherapy

Melanoma patients with primary lesions <1.5 mm in thickness have about a 20% relapse rate, those with lesions >1.5 mm and <3 mm have an approximately 50% relapse rate, and those with nodal metastases have an approximately 60–80% relapse rate, depending upon whether clinical or pathological

Table 5. Multidrug regimen: miscellaneous combination chemotherapy

Agents	Response	Author	Year
DTIC, fotemustine	33.3% (28.6% cerebral mets)	Avril	1990 [62]
CDDP, ifosfamide	40%	Verweij	1990 [63]
CARBO, cytosinearabinoside	37%	Jeremic	1991 [64]
DTIC, videsine	25%	Ringbor	1989 [65]
CDDP, cytarabine (ARA-C)	16%	Bejetta	1986 [66]
DTIC, TAM	28%	Cocconi	1992 [17]
DTIC, ACT-D	29%	Costanzi	1976 [67]
DTIC, procarbazine	17%	Einhorn	1974 [68]
		Wittes	1978 [69]
Vinblastine, BLEO, CDDP	58%	Nathanson	1980 [70]
DTIC, VA, anthracycline	39%	Chauvergne	1978 [71]
CCNU, VCR, BLEO	48%	DeWasch	1976 [72]
DTIC, fotesmustine, vindesine	43%	Khayat	1992 [73]
DTIC, BLEO, vindesine	32.2%	Mulder	1989 [74]
Procarbazine, VCR, CCNU (POC)	48%	Carmo-Pereira	1980 [75]
DTIC, CARBO, TAM	23%	Ferri	1992 [76]
Carmustine, 6-thioguanine	17%	Morton	1987 [77]
CDDP, vinblastine	8%	Creagan	1987 [78]
VCR, CCNU, procarbazine, cyclophosphamide (POCC)	33%	Green	1980 [79]
Cyclophosphamide, oncovin, methyl-CCNU, BLEO (COMB)	18%	Livingston	1975 [80]
DTIC, BCNU, actinomycin D, VCR	17%	Creagan	1986 [81]
CDDP, ifosfamide	43%	Paleske	1987 [82]
CDDP, actinomycin D	25%	Paleske	1987 [82]

staging is used and the number of nodes. Thus the use of chemotherapy as an adjuvant in high-risk primary and node-positive melanoma has been a subject of interest for many years. Innumerable studies have been reported using chemotherapy, immunotherapy, or a combination of the two. This discussion is restricted to chemotherapy studies.

Although occasional prospective random chemotherapy studies have demonstrated efficacy, most have not. Table 6 [86–93] lists these trials. The study of Bonzet [86], suggesting the superiority of a four-drug chemotherapy program, has too few patients to be reliable. The Karakousis [92] study contains a heterogenous group of patients, which may or may not have biased the study due to maldistribution in the study groups. The study of Ghussen [91] has a rather small number of patients, mainly with lower extremity primary melanoma, distributed in several stages, and utilizes hyperthermic perfusion with melphalan. An attempt to reproduce this study with larger number of patients is currently in progress (WHO and North American Perfusion Group). In addition, levamisole (not strictly a cytotoxic agent) was proposed in the NCI Canada study to have efficacy as an adjuvant similar to that shown in colon cancer [93]. This trial, however,

Table 6. Prospective randomized trials of adjuvant chemotherapy

Eligibility	No. of patients	Treatments	Results	Author	Year
			% DFS at 2 yrs		
Clinical stage I,	28	Control	66	Banzet	1978 [86]
(Clark III–V),	24	Chemotherapy	82.0		
axial location			$p < 0.05$		
			Median DFS		
Stages I, II, III	84	Control	73 wks	Hill	1981 [87]
	81	DTIC	40 wks		
			Median DFS		
Stage I, Clark 4	41	Control	13 mo	Fisher	1981 [88]
(>2.25 mm), 5	40	Methyl-CCNU	30 mo		
Stage II			$p = 0.068$		
Stage I, Clark	185	Control	30.4 ± 8.3	Veronesi	1982 [89]
3–5	192	DTIC	37.2 ± 7.9		
			% DFS at 5 yrs		
Stage I, Clark 3	96	Control	58	LeJeune	1988 [90]
(>1.5 mm), 4	77	DTIC	50		
or 5					
			Recurrence rate		
Stage I, II	54	Control	48%	Ghussen	1988 [91]
	53	Hyperthermic perfusion + MEL	11% ($p = 0.0001$)		
			% DFS at 2 yrs		
Stage I, II, III	70	Control	24	Karakousis	1989 [92]
	65	BCNU + VCR + Act-D	38		
			$p = 0.034$		
			% DFS at 5 yrs		
			% *p*		
Stage I	137	Observation	55 —	Quirt	1991 [93]
Clark III, IV, V	135	Levamisole	66 0.07		
Satellites	135	Levamisole + BCG	58 0.75		
Nodal	136	BCG	50 0.40		

After Mastrangelo. 1991. stage I = primary; II = regional node positive; III = distant metastases.

showed marginal statistical significance and has not been confirmed by other investigators.

Similarly, the use of alpha-interferon as an adjuvant (see Chapter 9) is under evaluation in a prospective random study carried out by ECOG (intergroup study). Currently, however, the clinician is left in the frustrating position of not having rigorous clinical trials data that has been replicated and that demonstrates clear-cut efficiacy of a specific regimen used in an adjuvant setting for high-risk primary or regional nodal metastatic melanoma. In selected patients, particularly those who are young and have very high-risk disease (primary thickness >3.0 mm and/or clinical or pathological

intransit or regional node metastases), it is the author's view that the use of chemotherapy with an active advanced disease regimen is justified. All patients who are eligible should be put in appropriate adjuvant studies.

5. Regional chemotherapy

Although melanoma tends to present with showers of micrometastases, and therefore frequently manifests multiple regional or distant metastatic sites simultaneously, the use of regional therapy is sometimes justified [94]. Examples include extremity primaries (whether locally recurrent or in the adjuvant setting), individuals who present with single visceral metastatic sites (e.g., liver metastases in ocular melanoma), pleural effusions, and leptomeningeal metastases or cerebral metastases [95].

Perfusion of patients with extremity primary disease is an outstanding example of regional therapy, and is dealt with in Chapter 11 (also see Coit [96]). A summary of results with regional arterial therapy is seen in Table 7 [36,97–104]. Drugs employed as intraarterial agents have included DTIC, cisplatin, and fotemustine. Although most intraarterial therapy is given in the extremities, it has also been used in the hepatic artery, especially together with polyvinyl or gelatin sponge embolization. Under such circumstances, a significant objective response rate can be achieved, and occasional complete responders are seen. When intraarterial therapy is given in the extremity, the results may be superior when external tourniquet control is

Table 7. Regional arterial infusion therapy

Agents	Tumor site	No. pts.	Responses	Response rate	Author	Year
DTIC + radiation	Leg	14	CR14	100%	Gundersen	1986 [97]
DTIC	Extremities, liver, vulva	17	1CR + 6PR	41%	Einhorn	1973 [98]
CDDP	Extremities	15	10PR	67%	Bland	1989 [99]
CDDP	Extremities, liver, brain	19	2CR + 2PR	21%	Calvo	1980 [100]
CDDP + DTIC	Extremities, trunk, unknown	30	3CR + 8PR	37%	Calabro	1990 [101]
DTIC + CDDP	Extremity, vulva	9	1CR + 3PR	44%	Frost	1985 [102]
CDDP + polyvinyl sponges	Liver	30	1CR + 13PR	46%	Mavligit	1988 [103]
CDDP + gelatin sponge embolization	Leg, bladder	3	1CR + 2PR	100%	O'Keefe	1989 [104]
Fotemustine	Hepatic mets	13	2CR + 6PR	61.5%	Khayat	1991 [36]

used in an attempt to minimize systemic spill of chemotherapeutic agents and subsequent systemic toxicity.

The use of intrapleural chemotherapy in melanoma is indicated where pleural effusion is a predominant or symptomatic metastatic manifestation. The treatment is similar to that of pleurodesis in other tumor types, utilizing bleomycin (30–60 mg), triethylen ethiophosphoramide (thiotepa 15–30 mg), tetracycline, or other agents. Experience with DTIC, cisplatin, and BCNU intrapleurally are limited. When such pleurodesis is carried out, it is important to emphasize that as much pleural fluid is removed as possible to limit drug dilution, drug must be left in the pleural space with a clamped chest tube for at least 24 hours, and repeat installation of chemotherapeutic agents into the pleural space may well increase the likelihood of control of pleural effusion.

Central nervous system metastatic disease in melanoma is extremely common. Intrathecal therapy is probably of little help in patients with parenchymal nodular metastatic lesions in the brain (see Chapter 13). The occasional appearance of leptomeningeal malignant melanoma without parenchymal lesions [105] has occasioned the used of intrathecal chemotherapy. The agent most widely used is DTIC, given in 5–20 mg mixed in 0.5–10 ml of saline plus CFS, to which 0.8 ml of sodium bicarbonate may be added to neutralize citric acid. Doses are delivered over a 10 minute period and are given approximately q 4 days up to six doses. Responses to this type of intrathecal treatment may be somewhat limited in duration.

Intralesional or topical chemotherapy has been used in melanoma in a number of settings. The agents used, including pyridoxal [106], IL-2, alpha-interferon, and transretinoic acid [107], are primarily in the area of immunotherapy and will be dealt with in Chapter 9. Fotemustine, which is a highly reactive chloroethyl-nitrosourea, has been used intralesionally for cutaneous and subcutaneous melanoma metastases, resulting in minor necrosis followed by total remission of the metastases [108].

6. Drug resistance

Melanoma is a disease that typifies the problem of primary (untreated), as well as secondary (prior chemotherapy), drug resistance. At least five major mechanisms of drug resistance have been postulated, which include:

1. P-glycoprotein (MDR mediated)
2. Glutathione (GSH)
3. Metallothionein
4. Glutathione transferase (GT)
5. Topoisomerase-II

In a series of studies by McClay et al. [109], clinical evidence of Tamoxifen enhancement of three-drug (DTIC, BCNU, cisplatin) chemotherapy has been suggested. Although McClay [50] demonstrated in vitro that Tamoxifen appeared to have a synergistic effect with DDP, clinical data suggest that

Tamoxifen may enhance the effectiveness of DTIC [17]. In studying the cisplatin-Tamoxifen interaction, McClay [50] was unable to demonstrate P-glycoprotein inhibition, increased uptake and retention of DDP, or a change in the levels of glutathione or metallothionein in the cisplatin-Tamoxifen treated cells. McClay was also unable to demonstrate similar synergistic interaction between Tamoxifen and BCNU. Whether Tamoxifen acts to alter primary or secondary drug resistance in melanoma cells is unclear at this time.

Studies of Yang and Horwitz [110,111] using a variety of in vitro systems have demonstrated inhibition of MDR and P-glycoprotein-mediated drug efflux. In addition, Fleming et al. [112] have demonstrated that progesterone inhibits the binding of drugs by P-glycoprotein and reverses MDR in vitro. Presumably antitumor agents that could be favorably affected by such a synergistic interaction would include the vinca alkyloids, anthracyclines, antitumor antibiotics, etoposide, taxol, and possible other drugs.

In an attempt to replicate this data in vivo, a recent study was undertaken by the authors in which patients were placed on a combination of BCNU, DTIC, and cisplatin using the standard regimen. In addition, megesterol acetate (Megace), a synthetic congener of progesterone, was given continuously at 40 mg qid PO. Megace was chosen because it also has antianorectic and anabolic effects in cancer patients. Partial and complete responses have been observed. (L Nathanson, Melanoma Research 3:36, 1993).

In addition to the above studies, Huang [113] has demonstrated that low levels of topoisomerase II expression are associated with doxorubicin-resistant human melanoma cells. Nair [114] has suggested that GSH-dependent detoxification enzymes may play an important role in low-level doxorubicin resistance in human melanoma cells. In another recent study by Lutsky [115], overexpression of P-glycoprotein appeared to be the dominant mechanism of resistance to vincristine, etoposide, actinomycin D, and taxol, rather than overexpression of GST or GSH levels.

Hanson [116] has attempted to overcome resistance to the cytotoxic effects of melphalan in human melanoma cells, utilizing the glutathione transferase inhibitor ethecrinic acide. This GST inhibition appeared to be effective in vitro.

Further work on multiple means of overcoming primary and secondary drug resistance must be sought in human malignant melanoma.

7. Future prospects

Primary resistance of melanoma to cytotoxic chemotherapeutic agents remains a perplexing and frustrating problem. Therapeutic attempts to exploit the unique differentiated property of the melanoma cell to produce pigment have been disappointing [117]. The use of newer chemotherapeutic agents (ifosfamide, taxol, fotemustine) is one approach to the solution of this

problem. New data utilizing possible inhibitors of primary or secondary drug resistance, such as Tamoxifen, Megace or inhibitors of GST-induced resistance (ethacrinic acid) suggest alternative approaches. Exploration of methods for preventing the development of CNS metastases (diminishing metastatic potential of tumor cells) should be an additional challenge for the future. Antiangiogenic factors are becoming available and melanoma, a tumor known for the development of an abundant but fragile tumor vasculature, could be an ideal model for the study of such agents.

The increasing incidence and high death rate from this malignancy demand a continuing pursuit of all of these avenues of research.

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13. Radiotherapy of melanoma

Michael J. Dougherty and Morton M. Kligerman

1. Introduction

This chapter discusses the radiobiology of melanoma as well as the clinical application of radiotherapy to patients with primary and metastatic disease. The treatment of ocular melanomas is addressed in Chapter 7.

2. Radiobiology

Traditionally, melanoma has been considered resistant to radiation. This resistance is likely multifactorial in nature. There is an extensive body of literature dealing with melanoma's radiobiology [1]. Laboratory investigations have included both in vitro and in vivo experiments. The current data suggest that there is significant heterogeneity between and amongst different tumors. This, in turn, accounts for the range of dose fractionation schemes that have been employed.

In vitro studies on melanoma cell lines have focused on a number of parameters, including cell survival curve characteristics as well as repair capacity. Cell survival curves are generally defined by the parameters D_o, D_q, and n (Fig. 1). D_o, or the slope of the straight line of the survival curve, is the dose that reduces cell survival to 37% of its original value and is a measure of cellular sensitivity. The extrapolation number, n, and the quasithreshold dose, D_q, are both measures of the shoulder width. The ability of the cell to repair sublethal damage is directly proportional to the width of the shoulder.

Early in vitro studies demonstrated large shoulders on the cell survival curves, which led to attempts to improve clinical outcome by increasing the dose per fraction. Barranco et al. [2] found an average n of 40 and a D_o = 125 in three human melanoma lines. Dewey [3] found an extrapolation number of 25 in experiments with the Harding-Passey melanoma line.

Since those early studies much data have accumulated regarding the radiobiology of melanoma. There is a wide range of variation, with D_o, D_q, and n values ranging from 0.67 to 2.11 Gy, 0.28 to 3.69 Gy, and 1.2 to 4.0,

L. Nathanson (ed.), Current Research and Clinical Management of Melanoma.

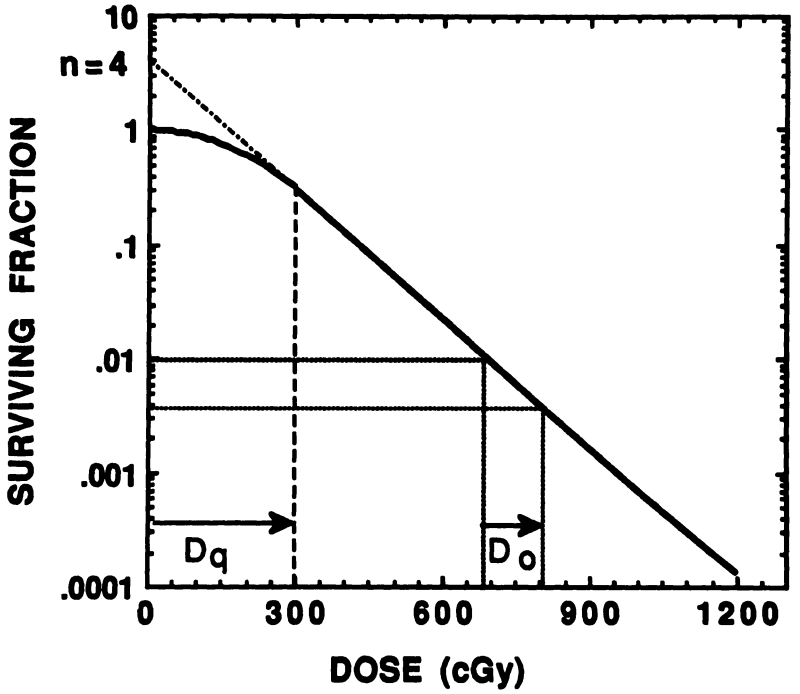

Figure 1. Cell survival curve. 'n' and 'D_q' are measures of the cell's ability to repair sublethal radiation damage. In the illustration D_q is equal to 100 cGy. The wider the D_q the greater is the cell's capacity to repair this damage. 'Do' is a measure of the sensitivity of the cells to radiation. It represents the amount of radiation that will reduce the number of cells to 37% of their original quantity. In the illustration the reduction is from 10^{-2} to 3.7×10^{-3} cells and equals 120 cGy. Since the D_o is on the exponential portion of the survival curve, every time 120 cGy are given, a further reduction by 37% occurs. When the exponential portion of the curve is angled more steeply to the left, the cells are more sensitive and the D_o will be smaller. With the exponential portion angled less steeply to the right, the cells are more resistant and the D_o is larger.

respectively [1]. These differences are comparable to those reported for many other tumors of varying clinical radiosensitivity, thus suggesting that in vitro survival curve parameters cannot fully explain clinical radiosensitivity [8]. Furthermore, Rofstad [1] has shown variation in the survival curves of different clones within the same xenograft, indicating the presence of heterogeneous subpopulations within given tumors. More recently, McBride et al. [5] have shown that melanoma cell lines with large extrapolation numbers are associated with structural abnormalities on chromosome 7.

In vivo cell survival curve analysis has yielded similar heterogeneity as the in vitro studies. In Rofstad's [1] review, the D_o, D_q, and n values ranged from 2.27 to 5.85 Gy, 2.17 to 9.23 Gy, and 1.9 to 42, respectively. Further in

vivo characterization of radiosensitivity have included TCD_{50} and growth rate analyses. The results of TCD_{50} assays, the dose required to control 50% of tumors, demonstrate a large range of values (29.6–67.8 Gy) [6,7]. These studies have been criticized because of the unknown role of the host immune response [8]. Using five different cell lines, Rofstad [8] did attempt to address this issue by comparing TD_{50} values, or the number of tumor cells required to produce tumors in half of the inoculation sites in two groups of mice. Group 1 was given 5 Gy whole body radiation before the inoculation of cells to reduce the host immune response. Group 2 was inoculated without any prior manipulation. In 2 of the 5 cell lines in the irradiated mice, significantly smaller TD_{50} values were obtained compared to the unirradiated controls, indicating a host immune response. In the three remaining cell lines this phenomenon was not demonstrated.

Specific growth delay gives an estimate of delay in volume doubling time by a given dose of radiotherapy compared to an unirradiated control [9]. Bristow and Hill [10] found specific growth delays of 2.4–3.0 with the B16-F1 cell line in athymic nude mice after 20 Gy in either one or 10 fractions. Similarly, Rofstad and Brustad [8] have shown that human melanoma tumor xenografts grown in athymic nude mice have specific growth delays after 15 Gy that vary by a factor of 5 (2–9.5). Additionally, specific growth delay was not found to correlate with Do but did correlate with the surviving fraction in vitro after 6 Gy as well as increasing vascular density. In their study, the volume doubling time ranged from approximately 3.5 to 17.5 days and was inversely associated with specific growth delay [8].

Radiosensitivity is determined, in part, by the repair of radiation-induced damage. Potentially lethal damage repair (PLDR), or damage whose repair can be determined by postirradiation environmental conditions, has been quantitated as the increased survival after delay in subculturing cells that were irradiated in plateau phase conditions [11]. Early in vitro work by Weichselbaum et al. [12] demonstrated up to a 48-fold recovery after 12 Gy for the C-143 melanoma line. Recovery increased with increasing fraction size and led the authors to question the utility of large fractions. Other investigators have also demonstrated PLDR in vitro as well as in vivo in melanoma cell lines [13–15], but to a smaller extent than that shown by Weichselbaum. Bristow and Hill [10], however, did not demonstrate PLDR in vivo with melanoma cell line B16-F1. Similarly, Hall and associates [16] showed no relationship between the extent of PLDR and the traditional radiosensitivity of a given tumor. In their studies the extent of repair of sublethal damage seemed to correlate more closely with radioresistant phenotypes. More recently, Weichselbaum and Beckett [17] have analyzed a number of head and neck squamous cell carcinomas with a known outcome after radiotherapy, as well as tumors generally considered radioresistant (e.g., melanoma lines U1-mel and C-32) [17,18]. In an attempt to elucidate the relative contributions of inherent radiosensitivity (D_o), repair of sublethal damage (n), and PLDR, cells were given an equitoxic dose (5–9 Gy) and

cell survival curve parameters as well as PLDR at 24 hours were determined. The range of D_o was from 107 to 234 cGy (mean = 154 cGy), and the range of n was from 1.0 to 4.1 (mean = 1.6) [18]. The radiosensitivity of the tumor lines fell within the range of normal fibroblasts [18]. Tumor cell lines with surviving fractions >0.1 in plateau phase 24 hours after irradiation were from patients who had failed treatment or from tumors considered to be radioresistant [17]. Since the probability of survival at 24 hours depends upon both initial cellular damage as well as repair, the therapeutic outcome of radiotherapy may not always easily be predicted by any one radiobiologic parameter.

In conclusion, malignant melanoma appears to be very heterogeneous in its radiosensitivity and thus there likely is not one optimal dose-fraction scheme.

3. The role of radiotherapy in the treatment of primary melanoma

Surgical excision is the preferred treatment of primary melanomas [19]. The role of radiotherapy is limited in these patients, with the possible exception of lentigo maligna (LM) and lentigo maligna melanomas (LMM). These lesions are usually found on the head and neck of older people and account for less than 10% of melanomas [20]. In LM, the malignant cells are found only in the epidermis, and if untreated up to 50% of patients will develop dermal invasion (LMM). However, lentigo maligna melanomas appear to be less aggressive than other melanomas, with only approximately 10% of LMM developing regional nodal metastases [21].

Kopf et al. of New York University [22] treated 16 patients with LM with definitive radiotherapy. Patients were treated with orthovoltage every 3–4 days, with 2000 rad fractions given five times. Eleven patients (75%) were controlled locally. Two who were controlled locally and one patient with a nodular recurrence developed distant metastases. Proppe [22] treated 35 patients with orthovoltage using a similar dose-fractionation scheme. He reported no metastases at follow-up, ranging from 2 to 12 years. Similarly, Braun-Falco [22] treated 68 patients and observed no recurrences or metastases in the patients where follow-up was available.

Harwood [21] reported on 23 patients with LM and 28 patients with LMM treated definitively at Princess Margaret Hospital. Patients were treated with orthovoltage (100–250 KV) with the field encompassing the lesion and a 1 cm margin with doses of 3500 rad in 1 week to 5000 rad in 3–4 weeks depending on the field size. Of 20 evaluable patients with LM, only 2 (10%) have developed a recurrence and both were salvaged with excision or reirradiation. Of the 28 patients with LMM, 25 were assessable with only two (8%) local failures. Both patients were salvaged with further surgery. One patient subsequently developed regional node metastases and was alive with lung metastases at the time of the report. Complete regression of the

lesions was seen over 1–24 months after treatment with a median of 8 months.

There is relatively little information available on the treatment of superficial spreading and nodular melanomas with radiotherapy. These lesions are preferentially managed by surgical excision since, historically, melanomas have been considered to be resistant to radiation. Adair [23] found only a 2.5% response rate to irradiation, although these patients generally had advanced disease or disease in locations not suitable for wide excision. Unfortunately, no details of irradiation technique were given. Tod [24] also characterized melanoma as 'highly radioresistant,' although she did report that 21 of 27 (78%) of patients with 'early' melanomas treated with radiotherapy were alive 2–5 years after treatment. Other authors have also commented on the radioresistance of melanoma [25], although Ellis [26] reported favorable results in patients treated at Sheffield Radium Centre in the 1930s. He treated 35 patients with a variety of primary and metastatic sites. The majority were treated with brachytherapy. Twelve patients (34%) exhibited a 'definite' response to treatment, thereby implying a spectrum of clinical radiosensitivity.

A summary of more recent results is presented in Table 1. The majority of the series report on small numbers of patients treated with a variety of dose-fractionation schemes. Overall local control rates vary from 33 to 100%. Hellriegel [27] reported on 95 patients treated definitively at Univer-

Table 1. Primary radiotherapy for nodular melanoma

Author	No pts	Total dose (cGy)	Fraction size (cGy)	Local recurrence (%)	Survival	Ref
Hellriegel	95	11,000	600	—	68% 5 yr. crude	27
Sealy	2 unresect.	4500 R+ hyperbaric oxygen	450 R	50	—	28
	2 residual after surgery			0	—	
Lobo	2 microscopic residual	6000–8000 cGy	200–270	50	1 pt. alive 58 mos after treatment	29
Johanson	3 microscopic residual	2400 +/– misonidozole	800	33	100% 1.25–2.5 yrs. after treatment	30
	3 gross residual			67	33% 2.5 yrs. after treatment	
Rounasville	8 microscopic residual	600–7700	180–1000	12	—	31

sity Roentgen Institute in Frankfurt, West Germany from 1935 to 1960. The primary received a 10–11,000 rad, split course with 60 KV photons. The regional lymph nodes received 4000–8000 rad with 250 KV photons or 15–20 Mev electrons. The crude 5-year survival of 68% was comparable to results obtained in patients treated by pre- (86%) or post-excisional (54%) radiotherapy.

Elsmann [32] recently reported on 83 patients with various types of melanoma treated with orthovoltage. Sixty-four (77%) patients had lentigo maligna melanomas, whereas the remaining patients had superficial spreading (12%), nodular (9%), or melanomas not further characterized (8%). Patients were referred for definitive radiotherapy because of medical contraindications to surgery, locally advanced disease, or patient refusal. Sixty patients had gross residual disease present prior to treatment, while 23 patients were treated after gross total excision. Patients were treated with 30–100 KV X-rays, with total doses of 48–105 Gy with fraction sizes ranging from 3.5 to 7 Gy. With a median follow-up time of 42 months, only two patients developed tumor recurrence in the irradiated volume. Only 8 of the 33 observed deaths were attributed to melanoma. The authors recommend excising any nodular component of the tumor prior to radiotherapy, which permits the use of more superficial x-rays. In addition to the report of Elsmann [32] on superficial spreading melanomas, Harwood et al. [33] treated six patients following tumor excision or biopsy with no local recurrences. One patient succumbed to metastatic disease.

In conclusion, surgery is the treatment of choice for primary melanomas. The role of radiotherapy is limited in the management of the primary, although radiation should be considered for patients who are medically inoperable, have lesions not suitable for excision because of the size or location, have close or involved margins of resection, or refuse surgery. As indicated above, the results of treatment for lentigo maligna and lentigo maligna melanoma are considerably better than for nodular and superficial spreading tumors.

4. The role of radiation therapy in the management of metastatic malignant melanoma

Melanoma has been known to spread to nearly every major organ. The most commonly involved sites include the skin, subcutaneous tissues, lymph nodes, lungs, liver, brain, and bone. The estimated frequency is 11–53%, which is considerably less than that reported in autopsy series [20]. Radiotherapy is most commonly used in the palliation of superficial lesions, as well as in brain and bone metastases. This section addresses the results of palliative radiotherapy in the treatment of the CNS, bone, and superficial lesions. Also, the role of adjuvant hyperthermia, fast-neutron therapy, and the controversy regarding dose-fraction size will be examined.

4.1 Cerebral metastases

Metastatic melanoma involving the brain is relatively common. Clinical series have revealed that this involvement occurs in 12–20% of patients, while autopsy data indicates that subclinical involvement is quite common, with reported rates of 36–54% [20]. For solitary metastases, surgical resection followed by radiotherapy is the treatment of choice. In patients with multiple metastases radiotherapy is the preferred treatment.

4.1.1. Results of treatment. The results of radiotherapy series and corresponding dose schedules are presented in Tables 2 and 3. The majority of patients (65–93%) had extracranial disease present at the time of diagnosis of brain metastases. Fifteen to 60% of patients had solitary metastases and 0–38% underwent craniotomy, with 13–29% undergoing complete gross resection. Overall symptomatic improvement ranged from 17% to 83%, and the median survival of all treated patients ranged from 1 to 5 months.

The role of large daily treatment fractions in patients with brain metastases remains uncertain. Strauss et al. [44] reported a 100% response rate in seven patients treated with fraction sizes >400 cGy compared to 23% in 13 patients treated with fractions of ≤400 cGy. Katz [36] confirmed the benefit of large fractions but only in patients with solitary brain metastasis. Other authors [33,45,46] have found no benefit with large fractions. Ziegler and Cooper [39] noted a 30% incidence of moderate to severe side effects in patients treated with 500–600 cGy fractions compared to 12% in patients receiving 300 cGy fractions, even though all patients received 3000 cGy total dose. One patient in each group required emergent craniotomy because of increased intracranial pressure.

Choi et al. [47] reported on 59 patients treated at the M.D. Anderson Hospital with a variety of accelerated fractionation schemes. All patients had either no evidence of extracranial disease or underwent complete

Table 2. Results of radiotherapy for patients with brain metastases: Patient characteristics

Study	No pts	% solitary brain metastases	% extracranial disease	% craniotomy (complete res)	Ref
Gottlieb	41	—	78	17	34
Carella	60	—	85	—	35
Katz	63	60	84	16 (13)	36
Vlock	46	—	93	15	37
Choi	194	53	77	21 (16)	38
Ziegler	72	50	74	22 (22)	39
Retsas	69	29	84	—	40
Rate	77	29	—	13 (13)	41
Davey	41	15	85	12	42
Stevens	129	46	65	38 (29)	43

Table 3. Results of radiotherapy for patients with brain metastases

Study	Dose per fraction (cGy)	Total dose (cGy)	Response rate (%)	Median survival months (range)	Ref
Gottlieb	300	3000	39	2.9 (1–15+)	34
Carella	200–1000	1000–4000	76 (31% complete)	2.5–3.5 (0–50+)	35
Katz	267–600	2000–4000	46	Sol. RT = 3.2 Sol. S & RT = 15 Mult. = 2.3	36
Vlock	125–600	885–5400	40	2.5–3 (0–40)	37
Choi	188–375	3000–4800	40 (21 major)	3	38
Zeigler	300–600	3000	63	Overall = 5 (0–57), Solitary = 6 Multiple = 3.5 Resected = 13.0	39
Retsas	450	2250	—	—	40
Rate	≤300 ≥400	—	—	3.5 (all pts.) Sol. S + RT = 9 Sol. RT = 4 Mult. RT = 3	41
Davey	400	2000	—	3	42
Stevens	200–600	2000–4500	—	5 (1–87+)	43

Sol. = solitary metastasis; Mult. = multiple metastases; S = surgery; RT = radiotherapy.

resection of brain metastases. The patients were treated twice daily to a total dose of 3000–3750 cGy in 10 fractions over 1 week, or to 3750–4800 cGy in 20 fractions over 2 weeks. There were no differences in response rates with the various fractionation schemes; however, patients treated in 1 week had a significantly improved mean duration of response (59 weeks) compared to patients treated in 2 weeks (24–27 weeks). These findings led the authors to conclude that overall treatment time is more important than fraction size. This conclusion can be questioned since patients treated in 1 week received higher daily fractions (300–375 cGy) compared to patients treated in 2 weeks (188–240 cGy).

In conclusion, there is no convincing evidence concerning the utility of large individual ractions. Consequently, 2000 cGy in five fractions in 1 week or 3000 cGy in 10 fractions in 2 weeks are as effective as more protracted courses or schemes utilizing large individual fractions.

4.1.2. Prognostic factors. Pretreatment factors that correlated with survival include performance status [36], neurologic status [38], absence of extracranial disease [38,43], and number of metastases [42,43]. Treatment-related prognostic factors include surgical resection [36,38,39,41,43,47], clinical response [48], radiographic response [40], radiotherapy fraction size [36], and overall radiotherapy treatment time [47].

Choi et al. [38] noted a median survival of 8.4 months in patients without extracranial metastases vs. 2.7 months in patients with extracranial metastases at the time of presentation. Similarly, Stevens et al. [43] noted a median survival of 7 months in the absence of extracranial disease, compared to 5 months in patients with concomitant extracerebral metastases. This difference was significant in univariate, but not multivariate, analysis. Ziegler and Cooper [39] found that patients with only cerebral metastases had a median survival approximately twice that of patients with extracranial disease. Other authors [45] have not confirmed the prognostic importance of extracranial disease.

Patients with solitary metastases appear to have a more favorable prognosis. Davey and O'Brien [42] noted that survival was inversely related to the number of metastases present. Stevens et al. [43], in an univariate analysis, reported that absence of extracranial disease, a solitary cerebral metastasis, and tumor resection predicted for improved survival. Patients with a solitary metastasis experienced an 8-month median survival as compared to 4 months in patients with multiple metastases ($p < 0.0001$). On multivariate analysis only complete tumor resection significantly predicted improved survival. Furthermore, progressive intracerebral disease has been reported to account for 57–76% of deaths in patients with brain metastases despite the high incidence of extracranial disease [35–38]. In addition Choi et al. [38] have shown that at autopsy 94% of treated patients had intracranial disease present at the time of death. It appears, therefore, that despite aggressive treatment, the majority of patients with intracerebral metastatic disease die as a result of their brain metastases. This observation forms the rationale for surgical resection of solitary metastases.

4.1.3. The role of resection. The role of surgery in the treatment of patients with brain metastases from malignant melanoma is controversial. The results of several retrospective series are summarized in Table 5. The majority of the patients undergoing craniotomy had solitary intracerebral metastases and were also less likely to have extracranial disease than patients treated by radiation therapy alone. Most series show an improved median survival when compared to series reporting on patients treated without surgical resection. Care must be taken when interpreting these results, however, given their retrospective nature and inherent biases in patient selection factors.

Hagen [49] reported on 35 patients who underwent resection of a solitary brain metastasis at Memorial Sloan-Kettering Cancer Center from 1972 to 1987. Nineteen patients received postoperative radiotherapy and 16 did not. The use of radiotherapy was decided upon by physician preference. Most patients treated with radiotherapy received 2400–4000 cGy in 200–300 cGy daily fractions. Although the median survival time was the same between the two groups, the patients who received radiotherapy had a significant

Table 4. Results of surgical resection of brain metastases

Author	No pts	% solitary mets in resected pts	Survival median (months)		Ref
			S	S + RT	
Katz	10	100		14.9	36
Choi	32	—		4	38
Rate	10	100		9	41
Ziegler	16	81		13	39
Hagen	35	100	8.3	6.4	49
Oredsson	32	100	13		50
Brega	13	38	10		51
Stevens	37 comp. resection	76		10	43
	12 part. resection	—		5	

S = surgery; RT = radiotherapy.

Table 5. Results of radiotherapy for patients with bone metastases

Study	No mets treated	Fraction size (cGy)	Total dose (cGy)	Response rate (% improved)	Ref
Hilaris	40	—	2000–8000	50	48
Katz	48	257–1000	1500–4000	77	36
Rate	39	—	1100–4000	85	41
Konefal	28	<200–>500	≤2000–4000	68	46
Rounsaville	28	—	—	86	31

longer interval to CNS relapse (27 vs. 6 months, $p < 0.05$) and a decreased CNS relapse rate (37% vs. 69%). Furthermore, 85% of the surgery-only patients died of neurologic causes compared to 24% of the patients receiving postoperative radiotherapy.

Patchell et al. [52] reported on a randomized trial of surgery plus radiotherapy vs. radiotherapy alone in the treatment of patients with solitary brain metastases. All patients had both MRI and CT scanning to exclude multiple intracranial metastases. Radiosensitive tumors (small-cell lung cancer, lymphoma, germ cell tumors, multiple myeloma, and leukemia) were excluded. Forty-eight patients, 77% of whom had primary lung cancer, were randomized between surgical removal followed by 3600 cGy whole-brain radiotherapy in 12 fractions, and biopsy-only followed by radiotherapy. Fifty-eight percent of the patients had extracranial disease at the time of randomization. The overall median survival was significantly longer in the patients undergoing surgery (40 weeks vs. 15 week, $p < 0.01$). In addition, the rate of local recurrence was significantly decreased in the surgical group (20% vs. 52%, $p < 0.02$). Patients in the surgical group also remained

functionally independent longer than those patients receiving radiotherapy (38 weeks vs. 8 weeks median, $p < 0.005$).

In conclusion, the information available would suggest that patients with solitary metastases may benefit from surgical resection followed by radiotherapy. If resection cannot be performed for medical, technical, or patient preference, then palliative whole-brain radiotherapy should be offered.

4.2. Spinal cord compression

Spinal cord compression secondary to metastatic melanoma is relatively uncommon. In a review of the experience at the Memorial-Sloan Kettering Cancer Center, melanoma accounted for only 5% of 130 cases reported [53]. Strauss et al. [44] reported responses in 2 of 4 patients, and Konefal et al. [46] reported responses in both patients treated with radiotherapy alone. Herbert et al. [54] reviewed the treatment of 38 sites in 35 patients with spinal cord or cauda equina compression from melanoma from over a 20-year span at the University of Pennsylvania. Patients received 500–4000 cGy in daily fractions of 200–800 cGy, with a median total dose of 2850 cGy. Surgical decompression was performed in 11 cases. The CR rate was 39% and the overall response was 85% in 28 sites evaluable 1 month after treatment. The response was durable in 81% of patients. The complete response rate was significantly higher in patients treated with ≥3000 cGy (62%) compared to those receiving less than 3000 cGy (20%). The response rates were not influenced by surgical decompression. However, the laminectomy patients had a longer median survival (19 weeks) than patients treated without surgery (9 weeks).

4.3. Osseous metastases

Osseous metastases occur in 11–17% of patients with metastatic melanoma [20]. Up to 80% of bony metastases are axial, with the vertebral bodies being the most common site. Patients with lytic metastases >2.5–3 cm in weight-bearing bones where the cortex is ≥50% destroyed, or in patients with pathologic fracture, internal fixation prior to radiotherapy is advisable [55].

The results of treatment are summarized in Table 6. The efficacy of radiotherapy appears to be excellent, with response rates varying from 50 to 86%. The median survival ranges from 2.5 to 4 months. Though less common, appendicular metastases have been reported to response more favorably than axial metastases [46]. There does not appear to be an improvement in response rate with large fraction size [31,41,56]. On the other hand, several series have reported improved response rates with doses greater than 3000 cGy [31,56]. In a multivariate analysis, Rate et al. [41]

Table 6. Results of radiotherapy with superficial metastases

Author	Site[a]	Response rate	Ref
Habermalz	S	67%	57
	LN	62%	
Overgaard	S.LN	73%	58
Trott	S,LN	45%	59
Katz	S	53%	56
	LN	53%	
Strauss	S	78%	44
Rounsaville	S	51%	31

[a] S = skin, subcutaneous; LN = lymph node.

found no significant association between the rate of palliation and fraction size, total dose, age, sex, location of metastases, chemotherapy, or interval to metastases.

4.4. Superficial metastases

Superficial metastases to skin, subcutaneous tissues, and lymph nodes are the most common site of metastatic disease in patients with melanoma, occurring in 42–59% and 50–75% of patients in clinical and autopsy series, respectively [20]. Nearly 40% of patients with metastatic disease confined to the skin, subcutaneous tissues, and lymph nodes will survive greater than 1 year, and a small number (<10%) will survive 5 years. Furthermore, these lesions appear to respond better to treatment than do metastases to visceral organs [20]. Therefore, aggressive treatment of these patients is justified. Provided they are easily resectable, surgical excision is the treatment of choice. Radiation therapy is an excellent option for patients with multiple recurrences or lymph node recurrences in sites that are not readily accessible (e.g., mediastinum, pelvis, retroperitoneum).

The results of radiotherapy treatment are summarized in Table 6. The majority of patients appear to have achieved a good palliative response to radiotherapy. The complete response rates vary from 8% to 59% [31,57,58,62]. Overgaard et al. [58] have shown that patients with a CR have greater than a 70% probability of remaining free of relapse in the irradiated volume. They also analyzed survival in 45 patients whose disease was limited to the treatment fields. Patients with persistent local control had a 56% three-year survival rate compared to 0% in patients not achieving long-term local control.

The majority of the literature indicates a benefit to the use of individual fractions ≥400 cGy. Habermalz and Fischer [57] reported the Yale experience of treating 44 skin or subcutaneous lesions. The response rate was 90% with

fraction sizes ≥600 cGy as compared to 0% with fractions ≤500 cGy. All five lymph node metastases responded with high-dose fractions (≥600), whereas only 3 of 8 lymph node metastases treated with ≤500 cGy responded.

In a review of the literature, Overgaard [60] reported on 618 cutaneous, lymph node, mucosal, and visceral (excluding brain) malignant melanoma lesions treated with radiotherapy. Forty-eight percent of the lesions achieved a CR. There was a significantly improved complete response rate for fraction size >400 cGy (59%) vs. ≤400 cGy (33%). There was also a correlation between tumor volume and local control. This observation has been confirmed by other investigators [30,61]. In a prospective randomized trial, Overgaard et al. [62] compared 900 cGy × 3 and 500 cGy × 8, given twice weekly to 35 cutaneous or nodal metastases in 14 patients. All patients had metastatic or inoperable recurrent disease. The overall response rate was 97% (34 of 35). The complete response rate was 69% and there were no differences between the two regimens.

The benefit of large-dose fractions has been questioned by several investigators [44,59]. Trott et al. [59] reported on 44 cutaneous or lymph node metastases in 38 patients treated with radiotherapy alone. Patients received 2700–7200 cGy in 4–32 fractions over 14–55 days. Twenty lesions (45%) remained locally controlled for at least 2 years. There was no difference in control between patients treated with <400 cGy fractions (47%) compared to ≥400 cGy fractions (40%). The authors conclude that therapeutic outcome was most dependent on the overall treatment time rather than fraction size. In contrast, in Overgaard's literature review there was no correlation between the overall treatment time and control rates [60]. Similarly, Rounsaville et al. [31] and Bentzen [63] found no association between the response rate and treatment time in patients treated for gross residual melanoma.

There does not appear to be a convincing dose-response relationship in patients treated for superficial metastases (Table 7). Although patients were treated with various dose fraction schemes with different overall treatment times, combining the results show similar overall response rates with total doses <3000 (69 of 112 = 62%) vs. ≥3000 cGy (83 of 132 = 63%).

There is relatively little information available regarding the complications of large-fraction radiotherapy in the treatment of melanoma. Overgaard et al. [62] noted several cases of moderate fibrosis in patients treated with 900 cGy fractions. Rounsaville et al. [31] noted two cases of moderate to severe fibrosis in 51 patients treated for gross residual disease. Both patients received 800 cGy × 3 over 21 days. Johanson et al. [30] have reported a 5% incidence of severe complications using an identical treatment scheme for 54 patients with nodular melanoma. There was one case of femoral neuropathy, one brachial plexopathy, and one case of chronic abdominal pain in the irradiated volume. Strauss et al. [44] observed one case of soft tissue breakdown in 45 patients receiving ≤400 cGy compared to three cases in 48 patients treated with >400 cGy.

In summary, the majority of the literature would support the use of large

Table 7. The influence of total dose on local control for superficial metastases

Author	Site[a]	Total dose (cGy)	Response rate (%)		Ref
			Complete	Overall	
Habermalz	S	<3000		60 (15/25)	57
	LN	<3000	0 (0/2)	0 (0/2)	
Overgaard	S	<3000	40 (4/10)	90 (9/10)	60
	LN	<3000	43 (3/7)	86 (6/7)	
Lobo	S,LN,B,Br,V	<3000		62 (23/37)	29
Katz	S	<3000		67 (2/3)	56
Rounsaville	S,LN,V	<3000	4 (1/28)	50 (14/28)	31
Habermal	S	≥3000		67 (14/21)	57
	LN	≥3000	27 (3/11)	82 (9/11)	
Overgaard	S	≥3000	21 (3/14)	57 (8/14)	60
	LN	≥3000	11 (2/18)	50 (9/18)	
Lobo	S,LN,B,Br,V	≥3000		76 (26/34)	29
Katz	S	≥3000		36 (4/11)	56
Rounsaville	S,LN,V	≥3000	13 (3/23)	57 (13/23)	31
Hornsey	S,LN,B,V	<3500		58 (11/190)	64
Doss	S,LN,B,Br,V	≤3500		32 (6/19)	61
Hornsey	S,LN,B,V	>3500		69 (22/32)	64
Doss	S,LN,B,Br,V	>3500		41 (9/22)	61

[a] S = skin, subcutaneous; LN = lymph node; B = bone; Br = brain; V = visceral.

daily fractions in patients with superficial metastases from malignant melanoma. However, great attention must be exercised in treatment planning regarding field location, beam energy, and the tolerance of irradiated structures to minimize complications. Thus, in the absence of a prospective randomized trial, large doses per fraction appear justified given possible improvement in local control, acceptable toxicity, and decreased time commitment.

5. Modification of the radiation effect

In an attempt to improve the response rate of melanoma, various investigators have utilized hyperthermia and fast neutron therapy. Heat is directly cytotoxic but it also has a radiosensitizing effect. Furthermore, hypoxic cells that are relatively resistant to ionizing radiation may be more sensitive to heat than aerobic cells. In a review of the literature, Overgaard [65] reported thermal enhancement ratios of 1.43–4.0 with response rates of 59–90% compared to 17–57% with radiation alone. However, uniform heating is hard to obtain and the author concluded that hyperthermia must be considered experimental.

Using neutron therapy, Catterall [66] reported 62% permanent local control in 48 patients treated to 87 tumor sites. Blake [67] treated 143 tumors in 78 patients. Ninety-seven percent of patients responded with a CR of 58%. Unfortunately, severe fibrosis or necrosis developed in 15% of the treated sites. Those patients treated with larger fields had a greater chance of developing local complications.

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Index