CUTANEOUS MELANOMA

Etiology and Therapy

Cover image: Stereotactic radiosurgery and stereotactic body radiotherapy plans for melanoma metastasis. See page 103, chapter 8 for details.

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Edited by

WILLIAM H. WARD

JEFFREY M. FARMA

Department of Surgical Oncology,
Fox Chase Cancer Center,
Philadelphia,PA, USA

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Edited by
William H. Ward, MD and Jeffrey M. Farma, MD
Department of Surgical Oncology, Fox Chase Cancer Center, Philadelphia, PA, USA

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FOREWORD

Despite recent advances in both diagnosis and treatment, cutaneous melanoma continues to represent a significant clinical challenge. In the United States, approximately 87,000 new melanomas will be diagnosed in 2017 and over 9,700 people are expected to die as a result of their disease. Unfortunately, the incidence of melanoma continues to rise. Although genetic risk is certainly involved, environmental risks are increasingly modifiable and, with enhanced outreach and knowledge sharing, a reduction of the incidence of cutaneous melanoma is a reasonable goal. Admittedly, melanoma is a preventable disease for many patients.

This book, edited by Drs. Farma and Ward, gathers a diverse and accomplished group of authors with expertise in both clinical and scientific aspects of melanoma development, diagnosis, and treatment. An exhaustive review of the entire spectrum of cutaneous melanoma is presented, including its epidemiologic background, diagnostic strategies, updated treatment approaches, and long-term management. Many of the therapeutic options developed in recent years have generated newfound enthusiasm among clinicians caring for patients with advanced disease. However, proper workup at the time of initial diagnosis is paramount, and patient outcomes can definitively suffer if the appropriate staging is compromised. As such, clinicians involved in the diagnosis or treatment of cutaneous melanoma must be familiar with the importance of available screening options, methods of clinical or histologic diagnosis, and the staging ramifications of disease discovery. This book provides a robust review of each of these elements and is recommended to anyone involved in the study or treatment of cutaneous melanoma.

Anthony J. Olszanski, RPh, MD
Vice Chair, Department of Hematology/Oncology
Director, Phase 1 Developmental Therapeutics Program
Director, Medical Oncology Melanoma Program
Fox Chase Cancer Center
Philadelphia, Pennsylvania
November, 2017
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PREFACE

As surgical oncologists working at a tertiary cancer center, we have seen major advances and recent changes in how we evaluate and treat patients with melanoma. Melanomas can present anywhere in the body and affect all age groups. We see all stages of melanoma and are seeing more advanced melanomas than ever before. Historically, surgery was the mainstay of therapy for most melanomas with few systemic options. However, through research advances and clinical trials, radiation and targeted immunotherapy have changed how we evaluate and manage melanoma patients. Nonetheless, there is much more that needs to be discovered through research and innovation in order to improve outcomes and increase awareness.

The purpose of this book is to provide an up-to-date, concise, multidisciplinary overview of the epidemiology, pathology, and current evidence-based treatment options for patients with all stages of melanoma. These aspects are presented in two sections: epidemiology and pathophysiology, and therapy and management. In section 1, five chapters provide a comprehensive overview of the epidemiology, heterogeneity, and biology of melanoma. In section 2, six chapters discuss the various treatment options and surveillance recommendations for afflicted patients.

We greatly appreciate the efforts of our internationally recognized melanoma contributing authors for their time, experience, and knowledge. We hope to provide helpful, relevant information for all physicians and researchers who may treat patients with melanoma in hopes of improving knowledge and improving the overall care of these patients.

William H. Ward, MD
Jeffrey M. Farma, MD, FACS
Department of Surgical Oncology
Fox Chase Cancer Center
Philadelphia, Pennsylvania, USA
November, 2017
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CONTRIBUTORS

ABRAR A. QURESHI, MD, MPH

Department of Dermatology, The Warren Alpert Medical School, Brown University, Providence, RI, USA; Department of Epidemiology, Brown University School of Public Health, Providence, RI, USA; Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA; Department of Dermatology, Rhode Island Hospital, Providence, RI, USA

ADRIANA FREITAS NEVES, PHD

Institute of Biotechnology, Molecular Biology Laboratory, Universidade Federal de Goias, Catalao, Brazil

ALSHIMAA MOHAMED MOSTAFA, MSC

Department of Dermatology, Faculty of Medicine, Beni-Suef University, Egypt

AYMAN EL-MEGHAWRY EL-KENAWY, PHD

Department of Molecular biology, Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Egypt; Deptartment of Pathology, College of Medicine, Taif University, Saudi Arabia

BIRGIT BELTER, PHD

Helmholtz-Zentrum Dresden-Rossendorf, Institute of Radiopharmaceutical Cancer Research, Department of Radiopharmaceutical and Chemical Biology, Dresden, Germany

CARADEE Y. WRIGHT, PHD

Environment and Health Research Unit, South African Medical Research Council and Department of Geography, Geoinformatics and Meteorology, University of Pretoria, Pretoria 0001, South Africa

CAROLINA CONSTANTIN, PHD

Victor Babes National Institute of Pathology, Immunology Dept., Bucharest, Romania; Colentina University Hospital, Bucharest, Romania

CATHLEEN HAASE-KOHN, PHD

Helmholtz-Zentrum Dresden-Rossendorf, Institute of Radiopharmaceutical Cancer Research, Department of Radiopharmaceutical and Chemical Biology, Dresden, Germany

ELISABETH A. SEFTOR, BSC

Robert C. Byrd Health Sciences Center, Department of Biochemistry, West Virginia University, Morgantown, West Virginia 26506 USA; Cancer Institute, West Virginia University, Morgantown, West Virginia 26506 USA; Department of Biology, Shepherd University, Shepherdstown, West Virginia 25443 USA

EUNYOUNG CHO, SCD

Department of Dermatology, The Warren Alpert Medical School, Brown University, Providence, RI, USA; Department of Epidemiology, Brown University School of Public Health, Providence, RI, USA; Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

FERNANDO LAMBRETON, BS

Department of Surgical Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania; USA

JEFFREY M. FARMA, MD

Department of Surgical Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania; USA

JENS PIETZSCH, MD, PHD

Helmholtz-Zentrum Dresden-Rossendorf, Institute of Radiopharmaceutical Cancer Research, Department of Radiopharmaceutical and Chemical Biology, Dresden, Germany; Technische Universität Dresden, Department of Chemistry and Food Chemistry, Dresden, Germany

JIAN Q. YU, MD

Department of Diagnostic Imaging, Fox Chase Cancer Center, Philadelphia, Pennsylvania

KAREN TARASZKA HASTINGS, MD, PHD

University of Arizona College of Medicine Phoenix, 425 N. 5th St., Phoenix, AZ 85004, USA

KENNETH M JOYCE, MB BCH, BAO, MRCS, MCH

Department of Plastic & Reconstructive Surgery, Galway University Hospital, Galway, Ireland

MARIE LOUISE BØNNELYKKE-BEHRNDTZ, MD, PHD

Department of Plastic and Reconstructive Surgery, Aarhus University Hospital, Denmark

MARTIN A. WEINSTOCK, MD, PHD

Department of Dermatology, The Warren Alpert Medical School, Brown University, Providence, RI, USA; Department of Epidemiology, Brown University School of Public Health, Providence, RI, USA; Department of Dermatology, Rhode Island Hospital, Providence, RI, USA

MARY J.C. HENDRIX, PHD

Robert C. Byrd Health Sciences Center, Department of Internal Medicine, West Virginia University, Morgantown, West Virginia 26506 USA; Cancer Institute, West Virginia University, Morgantown, West Virginia 26506 USA; Department of Biology, Shepherd University, Shepherdstown, West Virginia 25443 USA

MARY NORVAL, PHD, DSC

Biomedical Sciences, University of Edinburgh Medical School, Edinburgh EH8 9AG, United Kingdom

MATTHEW P. RAUSCH, PHD

Surface Oncology, 50 Hampshire Street, 8th Floor, Cambridge, MA 02139, USA

MONICA NEAGU, PHD

Victor Babes National Institute of Pathology, Immunology Dept., Bucharest, Romania; Colentina University Hospital, Bucharest, Romania; University of Bucharest, Bucharest, Romania

NAIRA V. MARGARYAN, DVM, PHD

Robert C. Byrd Health Sciences Center, Department of Biochemistry, West Virginia University, Morgantown, West Virginia 26506 USA; Cancer Institute, West Virginia University, Morgantown, West Virginia 26506 USA; Department of Biology, Shepherd University, Shepherdstown, West Virginia 25443 USA

NATALIE H. MATTHEWS, MPHIL
Department of Dermatology, The Warren Alpert Medical School, Brown University, Providence, RI, USA

NEHA GOEL, MD
Department of Surgical Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania; USA

RICHARD E.B. SEFTOR, PHD
Robert C. Byrd Health Sciences Center, Department of Biochemistry, West Virginia University, Morgantown, West Virginia 26506 USA; Cancer Institute, West Virginia University, Morgantown, West Virginia 26506 USA; Department of Biology, Shepherd University, Shepherdstown, West Virginia 25443 USA

SNUR M. A. HASSAN, MSC
Department of Anatomy and Pathology, College of Veterinary Medicine, Sulaimani University, Kurdistan-Iraq

THAISE GONÇALVES DE ARAÚJO, PHD
Laboratory of Genetics and Biotechnology, Institute of Genetics and Biochemistry, Federal University of Uberlandia, Patos de Minas, Brazil

TORBEN STEINICHE, MD, PROF. DR. MED
Department of Pathology, Aarhus University Hospital, Denmark

WEN-QING LI, PHD
Department of Dermatology, The Warren Alpert Medical School, Brown University, Providence, RI, USA; Department of Epidemiology, Brown University School of Public Health, Providence, RI, USA

WENYIN SHI, MD, PHD
Department of Radiation Oncology, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA 19107, USA

WILLIAM H. WARD, MD
Department of Surgical Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania; USA

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Section I

Epidemiology and Pathophysiology

1 Epidemiology of Melanoma

NATALIE H. MATTHEWS[1] • WEN-QING LI[1,2] • ABRAR A. QURESHI[1,2,3,5] • MARTIN A. WEINSTOCK[1,2,4,5] • EUNYOUNG CHO[1,2,3]

[1]Department of Dermatology, The Warren Alpert Medical School, Brown University, Providence, RI, USA; [2]Department of Epidemiology, Brown University School of Public Health, Providence, RI, USA; [3]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA; [4]Center for Dermatoepidemiology, Department of Veterans Affairs Medical Center, Providence, RI, USA; [5]Department of Dermatology, Rhode Island Hospital, Providence, RI, USA

Author for correspondence: Natalie H. Matthews, Mphil, Department of Dermatology, The Warren Alpert Medical School, Brown University, Providence, RI, USA. E-mail: natalie_matthews@brown.edu

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Abstract: Melanoma is a potentially lethal cancer that is most commonly cutaneous. The worldwide incidence of melanoma has risen rapidly over the course of the last 50 years. Its incidence is greatest among fair-skinned populations, and in regions of lower latitude. Incidence is greater among geriatric populations, but melanoma is also among the most common cancers found in adolescent and young adult populations. In fact, it is one of the leading cancers in average years of life lost per death from disease. Melanoma incidence varies by sex, which is also associated with differences in melanoma anatomic site. Similar differences by region, ethnicity, age, and sex are observed in mortality rates of melanoma. In the setting of rising incidence and mortality, melanoma bears a heavy health and economic burden. Attributable costs are several billion in nations with greater melanoma incidence. Preventative strategies have been implemented in multiple high-risk regions with variable success. It is imperative that research efforts

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achieve better understanding of the risk factors and etiology of disease, with the goal to halt and reverse the progressive trend of rising incidence and mortality from melanoma.

Key words: Epidemiology; Incidence, Melanoma; Mortality; Prevention

Introduction

Melanoma is a malignant tumor that arises from uncontrolled proliferation of melanocytes—pigment-producing cells (1–4). While the most common form of melanoma is cutaneous, it can also arise in mucosal surfaces, the uveal tract, and leptomeninges. This chapter will focus on cutaneous melanoma.

Malignant melanoma is the most lethal form of skin cancer (5–7). Historically, melanoma was a rare cancer, but in the last 50 years its incidence has risen faster than almost any other cancer (8–11). In 2017, approximately 87,110 individuals are predicted to be diagnosed with melanoma in the United States alone (6, 9). While it still represents less than 5% of all cutaneous malignancies, melanoma accounts for the majority of skin cancer deaths (5, 6, 9). If melanoma is diagnosed in its early stages, resection of the lesion is associated with favorable survival rates. However, melanoma is an aggressive malignancy that tends to metastasize beyond its primary site (7, 12). Once melanoma is advanced, surgery is no longer sufficient and the disease becomes more difficult to treat (7, 12–14). Long-term prognosis after metastasis is grim; median survival with treatment, including treatment with immunotherapeutics like Ipilimumab, ranges from 8 to 12 months (6, 13, 14). However, more recently developed combined immunotherapeutic treatments with radiation can improve survival further to several years (13).

In addition to the considerable burden to public health, the annual costs of melanoma management are substantial (15). In the United States alone, the annual costs of melanoma treatment have risen by 288% in less than a decade. As new expensive pharmacologic treatments come to market, costs will likely rise at even greater rates. Melanoma comprises $3.3 billion of the total $8.1 billion in all direct skin cancer annual costs (16). Indirect costs associated with melanoma are estimated to be as high as over $3.5 billion annually (17). As incidence and mortality rises, costs for treatment and indirect care are projected to concurrently rise (15). However, as more preventative strategies are implemented to combat rising incidence, melanoma-related costs may improve with the potential of cutting economic burden by $2.1 billion a year (11).

To combat this cancer, population-based strategies have been implemented to reduce incidence through prevention (18). Epidemiological study has allowed investigators to better characterize which populations are most greatly affected, how they are affected, and what can be done to modify and improve upon prevention, treatment, and management strategies. Incidence, prevalence, and mortality studies reflect health and economic burden of disease. Incidence and prevalence includes all individuals that will and currently are receiving treatment, management, monitoring, and disability services as a result of their melanoma. These statistics underscore the demand and challenges of melanoma prevention and care,

as well as the continued need for epidemiologic surveillance. The aim of this chapter is to highlight changing trends in melanoma occurrence and mortality, and how these rates have influenced or been influenced by prevention strategies.

Incidence

Worldwide incidence of melanoma has steadily increased over the last several decades (5, 9–11, 19, 20). Annual incidence has risen as rapidly as 4–6% in many fair-skinned populations that predominate regions like North America, Northern Europe, Australia, and New Zealand (10, 21–33). Increases in incidence rates vary considerably across populations of different ethnicity and geographical location, and even within populations across age and gender (6, 7, 9, 19, 34, 35). These differences are important to consider to avoid masking true trends in melanoma incidence.

ETHNICITY

Melanoma demonstrates greater variation in incidence rates across different ethnic groups than that of most cancers (9). Melanoma is disproportionally reported among fair-skinned Caucasian populations (6, 9, 36, 37). This variation is partly attributable to decreased photoprotection from reduced melanin (38). The increased melanin barrier in darker-pigmented individuals decreases both ultraviolet (UV) A and B radiation through the skin (38–40). UV radiation is known to induce both cell death and malignant transformation of skin cells; it is considered the paramount risk factor for melanoma (41–46). Compared to fairer-skinned people, UVB radiation through the epidermis is diminished by approximately 50% in darker-skinned people (38), and UVA transmission through the dermis decreases from 27 to 4% at 314 nm and 47 to 14% at 400 nm (39).

Within the United States, differences among melanoma incidence by race are well-illustrated (11). The United States has a comprehensive cancer database. The United States Cancer Statistics (USCS) provides official federal cancer incidence statistics in 49 states and the District of Columbia (99.1% of the US population) using data from the National Program of Cancer Registries and the Surveillance, Epidemiology, and End Results (SEER) program (6). Of the 65,647 melanomas reported in the United States from this database, the overall annual age-standardized rate (ASR) of melanoma incidence was 19.7:100,000 cases (11). Non-Hispanic Caucasians accounted for the greatest incidence of ASR at 24.6:100,000 cases, followed by American Indian/Alaska Natives at 4.3, then Hispanics at 4.2, Asian/Pacific Islanders at 1.3, and lastly African Americans at 1.0 (6, 11). Although melanoma does disproportionally affect Caucasian populations, the incidence of disease can vary considerably depending on the geographic location of the population (47).

GEOGRAPHY

Incidence of melanoma varies by geographic location among people of the same ethnicity (47–50). Differences in geography can translate to differences

in atmospheric absorption, latitude, altitude, cloud cover, and season—all variables that influence incident UV radiation. In 1956, Lancaster found increasing melanoma mortality rates with increasing proximity to the equator, a phenomenon he termed the "latitude gradient" (47, 49, 51). Since then, similar trends of melanoma incidence have been reported around the world (Figure 1) (42, 48). In the lowest latitudes, melanoma annual incidence ASRs tend to be higher than that of ASRs in higher latitudes (Figure 1) (9). In 2012, of the 184 countries evaluated by the International Agency for Research on Cancer (IARC), the highest reported incidence rates for melanoma occurred in New Zealand (ASR = 35.8:100,000 cases per year), followed closely by Australia (ASR = 34.9:100,000 cases per year) (9). The following countries with elevated incidence ASRs were all of higher latitudes, including Switzerland (20.3:100,000 cases per year), the Netherlands (19.4:100,000 cases per year), Denmark (19.2:100,000 cases per year), Norway (18.8:100,000 cases per year), and Sweden (18.0:100,000 cases per year) (Table 1) (9). While these countries are at high latitudes, a north–south gradient of incidence rates has been observed, even among the northernmost Scandinavian nations (9, 52, 53). Similar observations have been made among Caucasian populations in the United States (54, 55), New Zealand (56), and other nations (48).

In Australia, those who live closer to the equator, and thus have a higher degree of sun exposure, have higher incidences of melanoma (57). Queensland, a predominately tropical state in Australia (latitude 27°S) has higher melanoma rates than New South Wales (latitude 34°S) (24, 57, 58). An inverse latitude gradient is observed in Europe (59). Within Europe, melanoma incidence is 3- to 6-fold higher in northern countries in Scandinavia than in southern countries like Spain and Italy (Table 1) (9). The differing incidence rates between northern

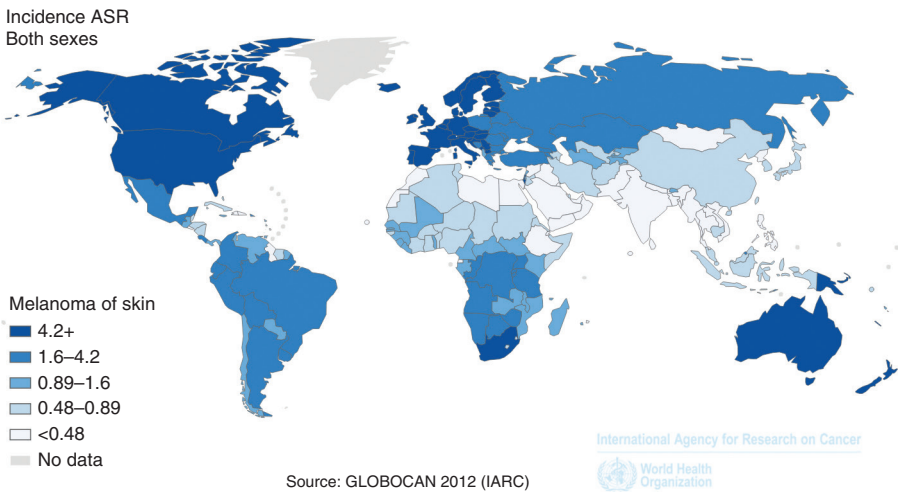

Figure 1 Worldwide melanoma age-standardized annual incidence rate by geography. Age-standardized rate (ASR) by world is expressed per 100,000 persons (9). Reproduced with permission.

and southern Europe could be partly attributed to different pigmentation characteristics that predominate the populations of each region. The fairer-skinned populations in Scandinavia and darker olive-skinned populations in southern Europe reflect patterns of melanoma incidence discussed previously about ethnicity (9).

Other European populations, such as those in the United Kingdom, Germany, Austria, and France, report melanoma ASRs in the range of 14.6–9.9:100,000 cases per year (Table 1) (9). The predominantly non-Caucasian populations of Africa, Asia, and the Pacific, and the mixed populations of Central and South America, consistently report melanoma rates less than 4:100,000 per year (Table 1) (9). Despite the geographic location of many Asian nations being near the equator, the incidence among Asians has remained largely unchanged and minimal. An unchanged Asian incidence, as with the case of the incidence in countries in Southern Europe and Africa, is likely attributed to a homogeneous darker-pigmented population (9).

TABLE 1 **Melanoma Annual Incidence by Worldwide Region and Country (N > 1000 persons) in Descending Order of ASR**

Population	N	Crude Rate	ASR (W)	Cumulative Risk
New Zealand	2473	55.4	35.8	3.9
Australia/New Zealand	14,738	53.8	35.1	3.75
Australia	12,265	53.5	34.9	3.72
Oceania	14,980	39.7	29.8	3.23
Switzerland	2484	32.1	20.3	2.05
The Netherlands	4804	28.7	19.4	1.95
Denmark	1596	28.5	19.2	1.91
Norway	1506	30.4	18.8	2.02
Sweden	2911	30.7	18	1.9
Northern Europe	23,311	23.2	14.6	1.51
United Kingdom	14,445	23	14.6	1.49
United States of America	69,109	21.9	14.3	1.55
Northern America	74,515	21.3	13.8	1.5
Czech Republic	2194	20.8	12.6	1.35
Finland	1208	22.4	12.6	1.34
Belgium	1941	18	12.1	1.19

Table continued on following page

TABLE 1 **Melanoma annual incidence by worldwide region and country (N > 1000 persons) in descending order of ASR** (Continued)

Population	N	Crude Rate	ASR (W)	Cumulative Risk
Western Europe	37,419	19.7	12.1	1.24
Israel	1111	14.4	11.4	1.26
Germany	16,884	20.6	11.4	1.2
Italy	10,012	16.4	11.4	1.09
France (metropolitan)	9871	15.6	10.2	1.04
Austria	1334	15.8	9.9	0.99
Canada	5382	15.5	9.6	1.02
More developed regions	191,066	15.3	9.6	1.01
Europe	100,442	13.5	8.6	0.89
Southern Europe	19,247	12.2	8.1	0.81
Hungary	1117	11.2	7.1	0.73
Serbia	1016	10.3	7.1	0.76
Spain	5004	10.7	6.9	0.7
Portugal	1101	10.3	6.7	0.66
South African Republic	1858	3.7	4.5	0.51
Central and Eastern Europe	20,465	7	4.5	0.49
Southern Africa	1924	3.3	4.2	0.47
Poland	2583	6.7	4.1	0.45
Russian Federation	8717	6.1	4.1	0.44
Ukraine	2792	6.2	4	0.44
Romania	1121	5.2	3.5	0.38
Colombia	1488	3.1	3.3	0.38
Micronesia/Polynesia	39	3.2	3.3	0.34
Micronesia	18	3.3	3.1	0.27
World	232,130	3.3	3	0.33
Argentina	1460	3.6	2.9	0.31
Brazil	6172	3.1	2.8	0.3
South America	10,956	2.7	2.5	0.27
Latin America and Caribbean	13,731	2.3	2.2	0.23
Turkey	1552	2.1	2.1	0.23
Mexico	2031	1.7	1.8	0.19

Table continued on following page

TABLE 1 **Melanoma annual incidence by worldwide region and country (N > 1000 persons) in descending order of ASR** (Continued)

Population	N	Crude Rate	ASR (W)	Cumulative Risk
Middle Africa	1085	0.8	1.7	0.22
Western Asia	3255	1.4	1.7	0.18
Central America	2403	1.5	1.6	0.17
Sub-Saharan Africa	6057	0.7	1.3	0.15
Central African Republic	32	0.7	1.2	0.15
Eastern Africa	1970	0.6	1.1	0.13
Middle-East and Northern Africa (MENA)	3830	0.9	1.1	0.12
Africa	6632	0.6	1.1	0.13
Less developed regions	41,064	0.7	0.8	0.08
Western Africa	1078	0.3	0.6	0.07
Japan	1371	1.1	0.6	0.06
China	9814	0.7	0.6	0.05
Eastern Asia	12,127	0.8	0.5	0.05
Asia	21,830	0.5	0.5	0.05
South-Eastern Asia	2354	0.4	0.4	0.05
Northern Africa	575	0.3	0.4	0.05
South-Central Asia	4094	0.2	0.3	0.03
India	2103	0.2	0.2	0.02
Western Sahara	0	0	0	0

Countries excluded from table include those with annual incidence <1000 persons per year (9).
ASR = Age-standardized rate (world), expressed per 100,000 persons. N = estimated annual incidence (9).

National figures allow for global comparisons of melanoma incidence. However, marked variances in melanoma incidence can be masked within countries that boast heterogeneous populations. These countries include the United States, New Zealand, Australia, Israel, and South Africa. Variances in melanoma incidence within a country can also be masked when nations span many degrees of latitude, like Australia. In all countries, melanoma rates are the highest among the fairest-skinned Caucasian residents (5, 9, 11). Conversely, lower incidence is seen among those with darker skin (6, 9, 11).

Differences in altitude have also been suggested to play a role in melanoma incidence. In countries with both high and low-latitude locations, regions of higher altitude have been associated with higher melanoma incidence (60–63). Similarly there are significantly higher incidence rates among individuals who

regularly partake in high-altitude activities like mountaineering (64). UV irradiance is associated with higher altitude. However, with higher altitude there are also changes in ozone absorption, decreased cloud cover, and increased surface reflectance from snow cover which can all also increase UV radiation.

AGE

Worldwide melanoma incidence ASRs climb steadily and peak at the seventh and eighth decades of life (Figure 2) (9). This trend is seen among most high-risk populations, including individuals in Australia and New Zealand (23, 65), and Northern Europe (28, 30). Incidence in the United States, however, peaks at the sixth decade of life (6). Americans aged between 55 and 74 comprise 44.9% of all diagnosed melanomas in the United States (6). While melanoma incidence is lower among people <40 years of age, it is one of the most common cancers diagnosed among adolescent and young adults (66, 67). In the United States, melanoma is the second most common cancer among women aged between 20 and 29 (6). Similarly, melanoma is among the most commonly diagnosed cancers in young adults worldwide (9, 68).

SEX

Melanoma affects women and men differently. This is in part reflected by differences in melanoma incidence by population (Figure 3) (9). When age is taken into account, adolescent and young adult women are more susceptible to melanoma than men (67–69). This may be in part due to the widespread use of indoor tanning among females, which is associated with increased melanoma risk (70–72). However, after the age of 40, rates reverse, and melanoma incidence among men is greater than that of women (6, 67–69). Overall, men are more susceptible to melanoma. Some posit that this increased susceptibility seen

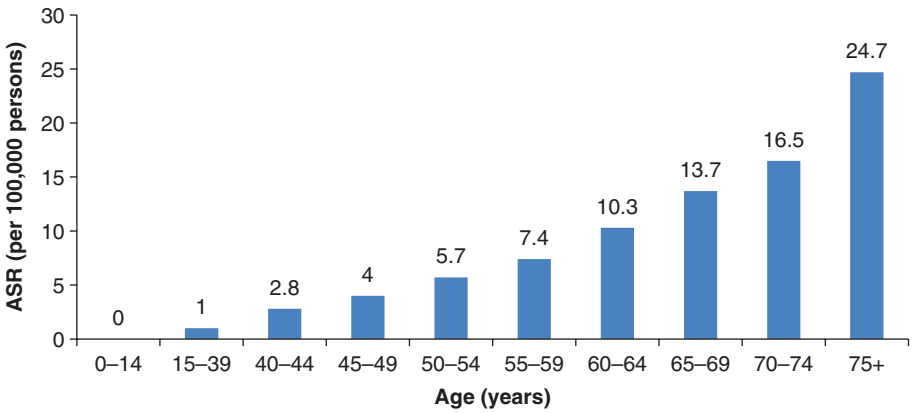

Figure 2 Worldwide age-standardized annual incidence of melanoma by age.
ASR = Age-standardized rate (world), expressed per 100,000 persons (9).

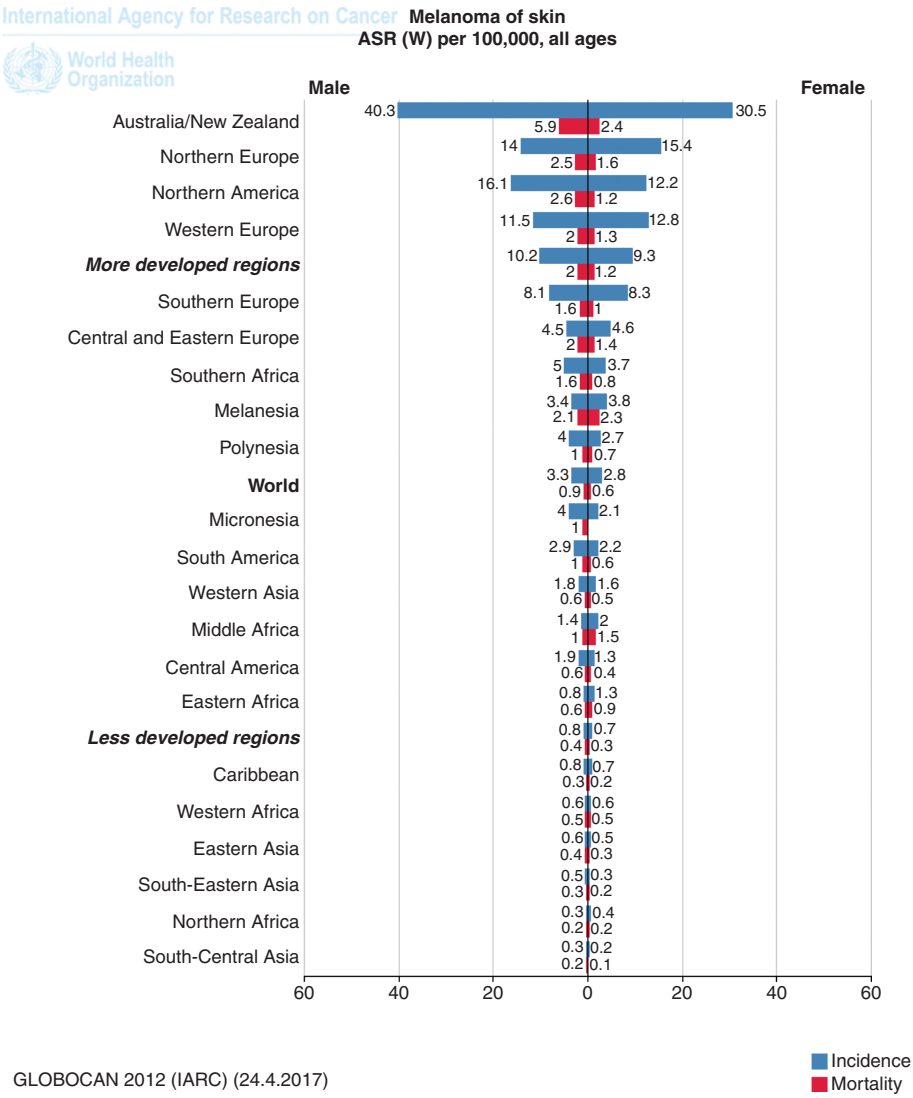

Figure 3 Melanoma age-standardized annual incidence and mortality rate by sex, world region, and development. ASR = Age-standardized rate (world), expressed per 100,000 persons (9). Reproduced with permission.

among men may be in part androgen driven (43, 72, 73). This difference in incidence by sex is exemplified in the United States with annual incidence ASRs of 29.2:100,000 cases in men compared to 17.3:100,000 cases in women (6). In fact, this increased incidence rate among men is observed across all ethnicities in the United States with ASRs (per 100,000 cases) of 33.1 for non-Hispanic Caucasian males and 19.9 for non-Hispanic Caucasian females, 5.0 for Hispanic

males and 4.7 for Hispanic females, 1.6 for Asian/Pacific Islander males and 1.2 for Asian/Pacific Islander females, and 1.1 for African American males and 1.0 for African American females (6). The only exception is among American Indian/Alaska Natives in which the ASR is 4.3:100,000 cases for men and 4.9:100,000 cases for women (6).

Historically, higher-latitude, lower-incidence populations in Scotland and Canada have reported substantially higher rates among females (27, 74). In Scotland, incidence of melanoma among females has been reported to be 2-fold higher than in males (74). Conversely, melanoma incidence is higher among men than women in most mid- to low-latitude populations like in the United States, Australia, and New Zealand (6, 9, 75). Overall, increases in melanoma incidence among men have since changed the lead women once had over men in high-latitude, low-incidence populations; men generally exceed women in these regions now (Figure 3) (9, 27, 28).

ANATOMIC DISTRIBUTION

Among Caucasian populations, melanoma is more frequently reported on the backs and shoulders of men and the lower limbs of women (76–80). For both sexes, given that these body site locations are associated with lower sunlight exposure, these findings have been used as supportive evidence for the intermittent UV exposure theory (81, 82). This theory posits that intermittent and intense sun exposure places individuals at increased risk for melanoma (81, 82). However, populations in low-latitude regions like Australia do not demonstrate similar patterns of distribution (83). Instead, Australians of both sexes most frequently report melanoma on high sun-exposed anatomic regions like their head and neck (83, 84). If risk of melanoma per unit area of skin is compared, the face is reported most frequently among both sexes (83). This calculation is made by adjusting for the surface areas of the body sites being compared (83). The next most frequently found sites for melanoma, when adjusting for surface area, are the shoulders, upper arms, and backs of women, and the shoulders and backs of men (83). The lowest rates of melanoma are found on the buttocks of both sexes, and the female scalp (76, 85).

Also, when considering age, melanomas that develop on the trunk occur more often in the fifth to sixth decades of life, whereas melanomas that develop in high UV-exposed body regions, like the head and neck, occur more commonly in the eighth decade (75, 86–89).

PREDICTIONS AND TRENDS

For decades, melanoma incidence has progressively risen and is projected to continue to rise across the world (5, 9–11, 19, 20). Conversely, mortality rates have not always followed a similar trend (6, 9). The diverging trends between melanoma incidence and mortality has led some to question whether there is a true melanoma epidemic, or rather if increases in incidence represent improved screening techniques (5, 90).

Those who argue that increased incidence is largely attributable to increases in diagnosis cite the high percentage of diagnosed melanoma *in situ* (6, 22, 91).

In the United States, the annual incidence of melanoma *in situ* is 9.5% (91). Some clinicians within the United States have suggested reclassifying nonmalignant diagnosis of melanoma to address, in part, inflated incidence (92). Increased number of biopsies has also been attributed to the increasing incidence of melanoma (92).

Some investigators suggest that increased screening and biopsy alone cannot account for the dramatic changes observed in incidence (5, 20). For example, in the United States, increases in melanoma incidence has been demonstrated across all melanoma thickness classifications, independent of socioeconomic status, which some argue is a surrogate marker for health care access and screening (5, 20). Similarly, others have found corresponding increases in incidence of aggressive melanoma subtypes, like nodular melanoma, and increases in later staged tumors (93). Taken together, these findings suggest that while increased diagnoses may play a role in increasing trends of melanoma incidence, there is also a true increase in incidence worldwide.

Mortality

Melanoma mortality trends are variable and, as with incidence, are influenced by geography, ethnicity, age, and sex (11, 19, 65, 67, 68, 94, 95). Melanoma mortality rates have marginally increased among fair-skinned populations (19, 32, 68, 96). Like with melanoma incidence, among fair-skinned populations, melanoma mortality rate is highest in low-latitude regions (Figures 3 and 4) (9). In high-risk regions like New Zealand, Australia, North America, and Europe, mortality rates historically increased until the 1980s (97, 98), peaked between 1988 and 1990, and then gradually maintained a slow increase (19, 32, 56, 68, 96, 99, 100). Over the last decade, mortality rate has steadily increased at 1.5% in the highest observed countries of New Zealand and Australia (19). In Scandinavia, mortality rate has also steadily increased over the last decade, with annual ASR in Norway at 6×10^{-5} per person and 4×10^{-5}/person in Sweden (19). In the United Kingdom, mortality rate has risen steadily at 1.59% per year (19). The US mortality rate has slowed to a 0.20% annual increase (19). Similar trends have also been reported in East Asian populations (101).

Within ethnically heterogeneous countries like the United States, variations in melanoma mortality among population subgroups have been observed (94). Non-Hispanic African Americans have lower cause-specific mortality than non-Hispanic Caucasians (HR = 0.7, 95% CI = 0.6–0.8) (94). However, after controlling for stage and site at diagnosis, gender, and age and decade of diagnosis, non-Hispanic African Americans fare worse than non-Hispanic Caucasians in cause-specific mortality (94). Overall, 5-year survival is lower for African Americans than non-Hispanic Caucasians as well (22, 26). In fact, over the last decade, 5-year survival rate has decreased among African Americans, whereas it has increased among Caucasian populations (22). Some attribute this discrepancy in ethnicity in part to socioeconomic inequities (94).

Discrepancies in age and sex are also observed in melanoma mortality rates (Figure 3) (6, 9, 33). Worldwide, males have greater mortality rates than

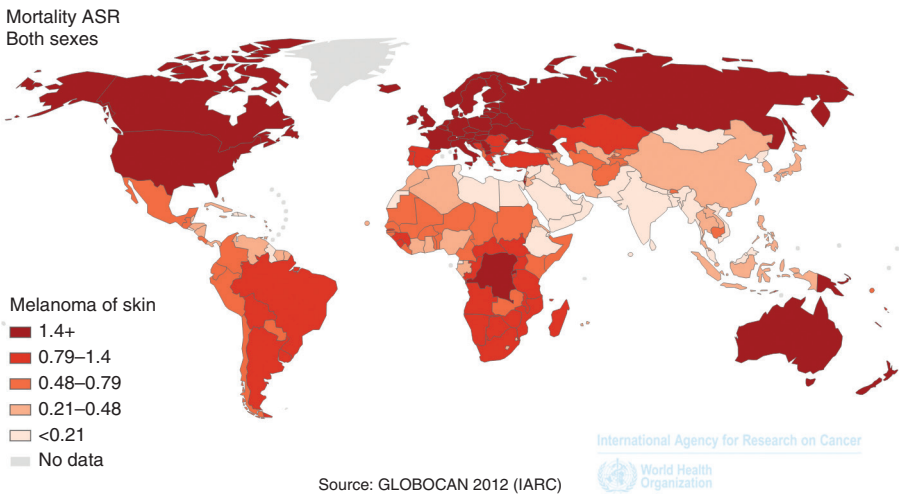

Figure 4 Worldwide melanoma age-standardized annual mortality rate by geography. Age-standardized rate (ASR) by world is expressed per 100,000 persons (9). Reproduced with permission.

females (Figure 3) (9, 33). In the United States, mortality is greater among men than in women of all races with the annual ASR of deaths being 4.0:100,000 cases in men compared to 1.7:100,000 cases in women (6). This increased incidence of ASR is observed in 4.3:100,000 cases for non-Hispanic Caucasian males compared to 1.8:100,000 cases for non-Hispanic Caucasian females and 1.4:100,000 cases for American Indian/Alaska Native males and 0.5:100,000 for American Indian/Alaska Native females (6). ASR is 1.0:100,000 cases for American Hispanic males and 0.6:100,000 cases for American Hispanic females; 0.5:100,000 cases for African American males and 0.4:100,000 cases for African American females; and 0.4:100,000 cases for American Asian/Pacific Islander males and 0.3:100,000 cases for American Asian/Pacific Islander females (6). Similarly, with annual melanoma incidence, annual melanoma mortality is the greatest among individuals beyond their seventh decade worldwide (9, 33). Conversely in the United States, mortality peaks at between ages 75 and 84, and then declines when individuals are aged >84 (6).

Prevention

In the setting of rising global incidence of melanoma, health agencies across nations with substantial burden of disease have launched campaigns that aim to promote prevention. Prevention strategies that have been implemented range from primary prevention methods to reduce sun exposure and enforce stricter labeling protocol for sunscreens (18, 102), to secondary prevention methods like full-body visual skin exams (103).

PRIMARY PREVENTION

Nationwide efforts to reduce UV exposure have been attempted with variable success across high-incidence countries like the United States, the United Kingdom, Norway, Australia, and New Zealand (19, 102, 104, 105). In the United States, the indoor tanning industry accrues $3 billion per year in profit (106). Attempts have been made by the US Surgeon General to promote sun protection polices like mandatory sun protection factor (SPF) labeling on sunscreens, recommendations for use of broad spectrum sunscreen SPF 15+, and discouraging indoor tanning. Despite more rigorous attempts to regulate indoor tanning, 7.8 million women and 1.9 million men still engage in tanning device activity (70, 102). In the United Kingdom, a nationwide campaign, SunSmart, was launched in 2003 in an effort to reduce the rapid rise in melanoma incidence (107). As part of their campaign, SunSmart highlighted UV reducing methods like wearing sunscreen with SPF 15+, wearing protective clothing and hats, and staying indoors during high incident UV hours (107). Despite concerted efforts, melanoma incidence in the United Kingdom has continued to increase (9). Population-based investigations found that British residents continued to partake in high-risk behaviors and that men from lower socioeconomic groups were at the greatest risk for UV exposure (107). In Norway and Sweden, national campaigns have also been launched in an effort to address rapid rises in melanoma incidence (104, 105). Similar to the United Kingdom and the United States, there has been limited and varied success with their preventative measures (104, 105). Australia, however, has demonstrated one of the most successful responses to nationwide melanoma prevention efforts (19, 108). Historically, Australia has had the highest melanoma incidence rates in the world (9). In the 1980s, the Australian government launched a massive melanoma education campaign, SunSmart (what the British later adopted for themselves). Sunsmart was integrated into primary school curriculums and permeated community forums and workplace training (108). In the decades since launching the SunSmart campaign, melanoma incidence rates in Australia have slowed, and within younger birth cohorts, incidence has even decreased (9, 19, 108). Australia is no longer the global leader in melanoma incidence (9).

SECONDARY PREVENTION

The predominant method of secondary prevention of melanoma is visual skin examination. Among the largest efforts to promote standardized screening, Germany launched the Skin Cancer Research to Provide Evidence for Effectiveness of Screening (SCREEN) in Northern Germany (109). After a year of implementing the program, a 48% reduction in melanoma mortality was found in SCREEN regions compared to neighboring communities (109). There was an overall decrease in mortality from 1.7 deaths per 100,000 cases to 0.9 deaths per 100,000 cases (109). This decrease in mortality was not observed in regions that did not implement SCREEN (109, 110). There are few other nationwide campaigns that promote secondary prevention. Australia has integrated bolstered screening measures as part of their overall screening campaign (103). In the United States, the national Preventative Services Task Force (USPSTF) deemed that there was insufficient data available to recommend visual skin exams (111). In a systematic evidence review, the Agency for Healthcare Research and Quality on the behalf of the

USPSTF examined >12,500 scientific abstracts and 450 articles and reported that they were unable to find any evidence on the potential harms or benefits of screening to conclusively make a recommendation (111). Continued efforts must be made to better understand prevention methods and their effects on melanoma incidence and mortality.

Conclusion

Global melanoma incidence and mortality continues to rise (9). While its incidence is over 10-fold lower than that of other skin cancers (6), its capacity to rapidly metastasize and affect younger patients makes melanoma a significant health and economic burden on society (5, 6, 21). In the United States alone, the estimated attributable health care cost in 2020 to melanoma is $4.58 billion (112, 113). Among high-risk populations, melanoma incidence will likely continue to rise in the geriatric subpopulation (6). However, incidence trends among younger individuals are hopeful, with some trends stagnating and some even decreasing in high-risk populations (19, 58, 103). In high melanoma incidence nations like Australia, melanoma rates have largely stabilized (9, 19). This is also true of mortality rates (9, 19). Hopefully, as these trends continue to be monitored, rates will decrease further. Although melanoma incidence has not slowed in other high-incidence regions like Northern Europe, a similar decrease should be expected to follow if continued concerted efforts are launched toward melanoma prevention campaigns. Nevertheless, continued surveillance is necessary. As the average age of nationwide populations is projected to increase in the United States, the United Kingdom, and Northern Europe, there may be a continued increase in melanoma incidence. It is imperative that primary and secondary methods of prevention are implemented and studied. National health campaigns can look to countries like Australia for examples of successful skin cancer prevention. Ultimately, preventative measures must take the cornerstone in melanoma control.

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References

1. Lerner AB, McGuire JS. Melanocyte-stimulating hormone and adrenocorticotrophic hormone. Their relation to pigmentation. N Engl J Med. 1964;270:539–46. http://dx.doi.org/10.1056/NEJM196403122701101
2. Abdel-Malek Z, Suzuki I, Tada A, Im S, Akcali C. The melanocortin-1 receptor and human pigmentation. Ann N Y Acad Sci. 1999;885:117–33. http://dx.doi.org/10.1111/j.1749-6632.1999.tb08669.x

3. Abdel-Malek Z, Swope VB, Suzuki I, Akcali C, Harriger MD, Boyce ST, et al. Mitogenic and melanogenic stimulation of normal human melanocytes by melanotropic peptides. Proc Natl Acad Sci U S A. 1995;92(5):1789–93. http://dx.doi.org/10.1073/pnas.92.5.1789
4. Tsatmali M, Ancans J, Thody AJ. Melanocyte function and its control by melanocortin peptides. J Histochem Cytochem. 2002;50(2):125–33. http://dx.doi.org/10.1177/002215540205000201
5. Linos E, Swetter SM, Cockburn MG, Colditz GA, Clarke CA. Increasing burden of melanoma in the United States. J Investig Dermatol. 2009;129(7):1666–74. http://dx.doi.org/10.1038/jid.2008.423
6. Surveillance, Epidemiology, and End Results (SEER) Program Cancer Statistics Review, 1975–2013, National Cancer Institute [Internet]. November 2015 SEER data submission [cited posted to the SEER web site, 2016 Apr]. Available from: http://seer.cancer.gov/csr/1975_2013/
7. Erdei E, Torres SM. A new understanding in the epidemiology of melanoma. Exp Rev Anticancer Ther. 2010;10(11):1811–23. http://dx.doi.org/10.1586/era.10.170
8. Rigel DS, Carucci JA. Malignant melanoma: Prevention, early detection, and treatment in the 21st century. CA Cancer J Clin. 2000;50(4):215–36; quiz 37–40. http://dx.doi.org/10.3322/canjclin.50.4.215
9. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. 2013 [cited 2017 Apr 6]. Available from: http://globocan.iarc.fr
10. Kosary CL, Altekruse SF, Ruhl J, Lee R, Dickie L. Clinical and prognostic factors for melanoma of the skin using SEER registries: Collaborative stage data collection system, version 1 and version 2. Cancer. 2014;120(Suppl 23):3807–14. http://dx.doi.org/10.1002/cncr.29050
11. Guy GP, Jr., Thomas CC, Thompson T, Watson M, Massetti GM, Richardson LC. Vital signs: Melanoma incidence and mortality trends and projections – United States, 1982–2030. MMWR Morb Mortal Wkly Rep. 2015;64(21):591–6.
12. Califano J, Nance M. Malignant melanoma. Facial Plast Surg Clin North Am. 2009;17(3):337–48. http://dx.doi.org/10.1016/j.fsc.2009.05.002
13. Filippi AR, Fava P, Badellino S, Astrua C, Ricardi U, Quaglino P. Radiotherapy and immune checkpoints inhibitors for advanced melanoma. Radiother Oncol. 2016;120(1):1–12. http://dx.doi.org/10.1016/j.radonc.2016.06.003
14. Goodson AG, Grossman D. Strategies for early melanoma detection: Approaches to the patient with nevi. J Am Acad Dermatol. 2009;60(5):719–35; quiz 36–8. http://dx.doi.org/10.1016/j.jaad.2008.10.065
15. Tripp MK, Watson M, Balk SJ, Swetter SM, Gershenwald JE. State of the science on prevention and screening to reduce melanoma incidence and mortality: The time is now. CA Cancer J Clin. 2016. http://dx.doi.org/10.3322/caac.21352
16. Guy GP, Jr., Machlin SR, Ekwueme DU, Yabroff KR. Prevalence and costs of skin cancer treatment in the U.S., 2002–2006 and 2007–2011. Am J Prev Med. 2015;48(2):183–7. http://dx.doi.org/10.1016/j.amepre.2014.08.036
17. Pollack LA, Li J, Berkowitz Z, Weir HK, Wu XC, Ajani UA, et al. Melanoma survival in the United States, 1992 to 2005. J Am Acad Dermatol. 2011;65(5 Suppl 1):S78–86. http://dx.doi.org/10.1016/j.jaad.2011.05.030
18. Seite S, Del Marmol V, Moyal D, Friedman AJ. Public primary and secondary skin cancer prevention, perceptions and knowledge: An international cross-sectional survey. J Eur Acad Dermatol Venereol. 2017;31(5):815–20. http://dx.doi.org/10.1111/jdv.14104
19. Whiteman DC, Green AC, Olsen CM. The growing burden of invasive melanoma: Projections of incidence rates and numbers of new cases in six susceptible populations through 2031. J Investig Dermatol. 2016;136(6):1161–71. http://dx.doi.org/10.1016/j.jid.2016.01.035
20. Erdmann F, Lortet-Tieulent J, Schuz J, Zeeb H, Greinert R, Breitbart EW, et al. International trends in the incidence of malignant melanoma 1953–2008—Are recent generations at higher or lower risk? Int J Cancer. 2013;132(2):385–400. http://dx.doi.org/10.1002/ijc.27616
21. Nikolaou V, Stratigos AJ. Emerging trends in the epidemiology of melanoma. Br J Dermatol. 2014;170(1):11–19. http://dx.doi.org/10.1111/bjd.12492
22. American Cancer Society. Cancer facts & figures 2017 [Internet]. Atlanta, GA: American Cancer Society; 2017 [cited 2017 Apr]. Available from: https://www.cancer.org/content/dam/

cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2017/cancer-facts-and-figures-2017.pdf
23. Coory M, Baade P, Aitken J, Smithers M, McLeod GR, Ring I. Trends for in situ and invasive melanoma in Queensland, Australia, 1982–2002. Cancer Causes Control. 2006;17(1):21–7. http://dx.doi.org/10.1007/s10552-005-3637-4
24. Marrett LD, Nguyen HL, Armstrong BK. Trends in the incidence of cutaneous malignant melanoma in New South Wales, 1983–1996. Int J Cancer. 2001;92(3):457–62. http://dx.doi.org/10.1002/ijc.1203
25. Jemal A, Devesa SS, Hartge P, Tucker MA. Recent trends in cutaneous melanoma incidence among whites in the United States. J Natl Cancer Inst. 2001;93(9):678–83. http://dx.doi.org/10.1093/jnci/93.9.678
26. Jemal A, Saraiya M, Patel P, Cherala SS, Barnholtz-Sloan J, Kim J, et al. Recent trends in cutaneous melanoma incidence and death rates in the United States, 1992–2006. J Am Acad Dermatol. 2011;65(5 Suppl 1):S17–25.e1–3.
27. Ulmer MJ, Tonita JM, Hull PR. Trends in invasive cutaneous melanoma in Saskatchewan 1970–1999. J Cutan Med Surg. 2003;7(6):433–42. http://dx.doi.org/10.1007/s10227-003-0159-0
28. MacKie RM, Bray CA, Hole DJ, Morris A, Nicolson M, Evans A, et al. Incidence of and survival from malignant melanoma in Scotland: An epidemiological study. Lancet (London, England). 2002;360(9333):587–91. http://dx.doi.org/10.1016/S0140-6736(02)09779-9
29. Lasithiotakis KG, Leiter U, Gorkievicz R, Eigentler T, Breuninger H, Metzler G, et al. The incidence and mortality of cutaneous melanoma in Southern Germany: Trends by anatomic site and pathologic characteristics, 1976 to 2003. Cancer. 2006;107(6):1331–9. http://dx.doi.org/10.1002/cncr.22126
30. Stang A, Pukkala E, Sankila R, Soderman B, Hakulinen T. Time trend analysis of the skin melanoma incidence of Finland from 1953 through 2003 including 16,414 cases. Int J Cancer. 2006;119(2):380–4. http://dx.doi.org/10.1002/ijc.21836
31. Lipsker D, Engel F, Cribier B, Velten M, Hedelin G. Trends in melanoma epidemiology suggest three different types of melanoma. Br J Dermatol. 2007;157(2):338–43. http://dx.doi.org/10.1111/j.1365-2133.2007.08029.x
32. de Vries E, Bray FI, Coebergh JW, Parkin DM. Changing epidemiology of malignant cutaneous melanoma in Europe 1953–1997: Rising trends in incidence and mortality but recent stabilizations in western Europe and decreases in Scandinavia. Int J Cancer. 2003;107(1):119–26. http://dx.doi.org/10.1002/ijc.11360
33. Shen W, Sakamoto N, Yang L. Melanoma-specific mortality and competing mortality in patients with non-metastatic malignant melanoma: A population-based analysis. BMC Cancer. 2016;16:413. http://dx.doi.org/10.1186/s12885-016-2438-3
34. Olsen CM, Neale RE, Green AC, Webb PM, Whiteman DC. Independent validation of six melanoma risk prediction models. J Investig Dermatol. 2015;135(5):1377–84. http://dx.doi.org/10.1038/jid.2014.533
35. Apalla Z, Nashan D, Weller RB, Castellsague X. Skin cancer: Epidemiology, disease burden, pathophysiology, diagnosis, and therapeutic approaches. Dermatol Ther. 2017;7(Suppl 1):5–19. http://dx.doi.org/10.1007/s13555-016-0165-y
36. Padovese V, Franco G, Valenzano M, Pecoraro L, Cammilli M, Petrelli A. Skin cancer risk assessment in dark skinned immigrants: The role of social determinants and ethnicity. Ethn Health. 2017:1–10. http://dx.doi.org/10.1080/13557858.2017.1294657
37. Chao LX, Patterson SS, Rademaker AW, Liu D, Kundu RV. Melanoma perception in people of color: A targeted educational intervention. Am J Clin Dermatol. 2017;18(3):419–27. http://dx.doi.org/10.1007/s40257-016-0244-y
38. Brenner M, Hearing VJ. The protective role of melanin against UV damage in human skin. Photochem Photobiol. 2008;84(3):539–49. http://dx.doi.org/10.1111/j.1751-1097.2007.00226.x
39. Everett MA, Yeargers E, Sayre RM, Olson RL. Penetration of epidermis by ultraviolet rays. Photochem Photobiol. 1966;5(7):533–42. http://dx.doi.org/10.1111/j.1751-1097.1966.tb09843.x
40. Grigalavicius M, Moan J, Dahlback A, Juzeniene A. Daily, seasonal, and latitudinal variations in solar ultraviolet A and B radiation in relation to vitamin D production and risk for skin cancer. Int J Dermatol. 2016;55(1):e23–8. http://dx.doi.org/10.1111/ijd.13065
41. Seebode C, Lehmann J, Emmert S. Photocarcinogenesis and skin cancer prevention strategies. Anticancer Res. 2016;36(3):1371–8.

42. Moan J, Grigalavicius M, Baturaite Z, Dahlback A, Juzeniene A. The relationship between UV exposure and incidence of skin cancer. Photodermatol Photoimmunol Photomed. 2015;31(1):26–35. http://dx.doi.org/10.1111/phpp.12139
43. Li WQ, Cho E, Weinstock MA, Mashfiq H, Qureshi AA. Epidemiological assessments of skin outcomes in the nurses' health studies. Am J Public Health. 2016;106(9):1677–83. http://dx.doi.org/10.2105/AJPH.2016.303315
44. Qureshi AA, Laden F, Colditz GA, Hunter DJ. Geographic variation and risk of skin cancer in US women. Differences between melanoma, squamous cell carcinoma, and basal cell carcinoma. Arch Intern Med. 2008;168(5):501–7. http://dx.doi.org/10.1001/archinte.168.5.501
45. Wu S, Han J, Laden F, Qureshi AA. Long-term ultraviolet flux, other potential risk factors, and skin cancer risk: A cohort study. Cancer Epidemiol Biomark Prev. 2014;23(6):1080–9. http://dx.doi.org/10.1158/1055-9965.EPI-13-0821
46. Wu S, Han J, Vleugels RA, Puett R, Laden F, Hunter DJ, et al. Cumulative ultraviolet radiation flux in adulthood and risk of incident skin cancers in women. Br J Cancer. 2014;110(7):1855–61. http://dx.doi.org/10.1038/bjc.2014.43
47. Lancaster HO. Some geographical aspects of the mortality from melanoma in Europeans. Med J Aust. 1956;43(26):1082–7.
48. Crombie IK. Variation of melanoma incidence with latitude in North America and Europe. Br J Cancer. 1979;40(5):774–81. http://dx.doi.org/10.1038/bjc.1979.260
49. Lancaster HO, Nelson J. Sunlight as a cause of melanoma; a clinical survey. Med J Aust. 1957;44(14):452–6.
50. Weinstock MA, Colditz GA, Willett WC, Stampfer MJ, Bronstein BR, Mihm MC, Jr., et al. Nonfamilial cutaneous melanoma incidence in women associated with sun exposure before 20 years of age. Pediatrics. 1989;84(2):199–204.
51. Elwood JM, Lee JA, Walter SD, Mo T, Green AE. Relationship of melanoma and other skin cancer mortality to latitude and ultraviolet radiation in the United States and Canada. Int J Epidemiol. 1974;3(4):325–32. http://dx.doi.org/10.1093/ije/3.4.325
52. Moan J, Dahlback A. The relationship between skin cancers, solar radiation and ozone depletion. Br J Cancer. 1992;65(6):916–21. http://dx.doi.org/10.1038/bjc.1992.192
53. Magnus K. Incidence of malignant melanoma of the skin in the five Nordic countries: Significance of solar radiation. Int J Cancer. 1977;20(4):477–85. http://dx.doi.org/10.1002/ijc.2910200402
54. Lee JA, Scotto J. Melanoma: Linked temporal and latitude changes in the United States. Cancer Causes Control. 1993;4(5):413–18. http://dx.doi.org/10.1007/BF00050859
55. Eide MJ, Weinstock MA. Association of UV index, latitude, and melanoma incidence in nonwhite populations—US Surveillance, Epidemiology, and End Results (SEER) Program, 1992 to 2001. Arch Dermatol. 2005;141(4):477–81. http://dx.doi.org/10.1001/archderm.141.4.477
56. Bulliard JL, Cox B, Elwood JM. Latitude gradients in melanoma incidence and mortality in the non-Maori population of New Zealand. Cancer Causes Control. 1994;5(3):234–40. http://dx.doi.org/10.1007/BF01830242
57. Baade P, Meng X, Youlden D, Aitken J, Youl P. Time trends and latitudinal differences in melanoma thickness distribution in Australia, 1990–2006. Int J Cancer. 2012;130(1):170–8. http://dx.doi.org/10.1002/ijc.25996
58. Iannacone MR, Youlden DR, Baade PD, Aitken JF, Green AC. Melanoma incidence trends and survival in adolescents and young adults in Queensland, Australia. Int J Cancer. 2015;136(3):603–9.
59. Armstrong BK. Epidemiology of malignant melanoma: Intermittent or total accumulated exposure to the sun? J Dermatol Surg Oncol. 1988;14(8):835–49. http://dx.doi.org/10.1111/j.1524-4725.1988.tb03588.x
60. Krishnamurthy S. The geography of non-ocular malignant melanoma in India: Its association with latitude, ozone levels and UV light exposure. Int J Cancer. 1992;51(2):169–72. http://dx.doi.org/10.1002/ijc.2910510202
61. Gerbaud L, Lejeune ML, Abou-Samra T, Doz M, Mathey MF, D'Incan M, et al. Epidemiological survey of melanoma in the Auvergne region (France): Is there an increased incidence in Auvergne? Eur J Epidemiol. 2003;18(4):331–5. http://dx.doi.org/10.1023/A:1023643219686
62. Aceituno-Madera P, Buendia-Eisman A, Olmo FJ, Jimenez-Moleon JJ, Serrano-Ortega S. [Melanoma, altitude, and UV-B radiation]. Actas Dermosifiliogr. 2011;102(3):199–205. http://dx.doi.org/10.1016/j.ad.2010.08.003

63. Haluza D, Simic S, Moshammer H. Temporal and spatial melanoma trends in Austria: An ecological study. Int J Environ Res Public Health. 2014;11(1):734–48. http://dx.doi.org/10.3390/ijerph110100734
64. Moehrle M, Garbe C. Does mountaineering increase the incidence of cutaneous melanoma? A hypothesis based on cancer registry data. Dermatology (Basel, Switzerland). 1999;199(3):201–3. http://dx.doi.org/10.1159/000018274
65. Sneyd MJ, Cox B. A comparison of trends in melanoma mortality in New Zealand and Australia: The two countries with the highest melanoma incidence and mortality in the world. BMC Cancer. 2013;13:372. http://dx.doi.org/10.1186/1471-2407-13-372
66. Ballantine KR, Watson H, Macfarlane S, Winstanley M, Corbett RP, Spearing R, et al. Small numbers, big challenges: Adolescent and young adult cancer incidence and survival in New Zealand. J Adolesc Young Adult Oncol. 2017;6(2):277–85. http://dx.doi.org/10.1089/jayao.2016.0074
67. Watson M, Geller AC, Tucker MA, Guy GP, Jr., Weinstock MA. Melanoma burden and recent trends among non-Hispanic whites aged 15–49 years, United States. Prev Med. 2016;91:294–8. http://dx.doi.org/10.1016/j.ypmed.2016.08.032
68. Garbe C, Leiter U. Melanoma epidemiology and trends. Clin Dermatol. 2009;27(1):3–9. http://dx.doi.org/10.1016/j.clindermatol.2008.09.001
69. Weir HK, Marrett LD, Cokkinides V, Barnholtz-Sloan J, Patel P, Tai E, et al. Melanoma in adolescents and young adults (ages 15–39 years): United States, 1999–2006. J Am Acad Dermatol. 2011; 65(5 Suppl 1):S38–49. http://dx.doi.org/10.1016/j.jaad.2011.04.038
70. Guy GP, Jr., Zhang Y, Ekwueme DU, Rim SH, Watson M. The potential impact of reducing indoor tanning on melanoma prevention and treatment costs in the United States: An economic analysis. J Am Acad Dermatol. 2017;76(2):226–33. http://dx.doi.org/10.1016/j.jaad.2016.09.029
71. Colantonio S, Bracken MB, Beecker J. The association of indoor tanning and melanoma in adults: Systematic review and meta-analysis. J Am Acad Dermatol. 2014;70(5):847–57.e1–18.
72. Zhang M, Qureshi AA, Geller AC, Frazier L, Hunter DJ, Han J. Use of tanning beds and incidence of skin cancer. J Clin Oncol. 2012;30(14):1588–93. http://dx.doi.org/10.1200/JCO.2011.39.3652
73. Li WQ, Qureshi AA, Ma J, Goldstein AM, Giovannucci EL, Stampfer MJ, et al. Personal history of prostate cancer and increased risk of incident melanoma in the United States. J Clin Oncol. 2013;31(35):4394–9. http://dx.doi.org/10.1200/JCO.2013.51.1915
74. MacKie R, Hunter JA, Aitchison TC, Hole D, McLaren K, Rankin R, et al. Cutaneous malignant melanoma, Scotland, 1979–89. Lancet (London, England). 1992;339(8799):971–5. http://dx.doi.org/10.1016/0140-6736(92)91539-K
75. Bulliard JL, Cox B. Cutaneous malignant melanoma in New Zealand: Trends by anatomical site, 1969–1993. Int J Epidemiol. 2000;29(3):416–23.
76. Osterlind A, Hou-Jensen K, Moller Jensen O. Incidence of cutaneous malignant melanoma in Denmark 1978–1982. Anatomic site distribution, histologic types, and comparison with non-melanoma skin cancer. Br J Cancer. 1988;58(3):385–91. http://dx.doi.org/10.1038/bjc.1988.225
77. Magnus K. Habits of sun exposure and risk of malignant melanoma: An analysis of incidence rates in Norway 1955–1977 by cohort, sex, age, and primary tumor site. Cancer. 1981;48(10):2329–35. http://dx.doi.org/10.1002/1097-0142(19811115)48:10%3C2329::AID-CNCR2820481032%3E3.0.CO;2-O
78. Popescu NA, Beard CM, Treacy PJ, Winkelmann RK, O'Brien PC, Kurland LT. Cutaneous malignant melanoma in Rochester, Minnesota: Trends in incidence and survivorship, 1950 through 1985. Mayo Clinic Proc. 1990;65(10):1293–302. http://dx.doi.org/10.1016/S0025-6196(12)62140-5
79. Masback A, Westerdahl J, Ingvar C, Olsson H, Jonsson N. Cutaneous malignant melanoma in south Sweden 1965, 1975, and 1985. A histopathologic review. Cancer. 1994;73(6):1625–30. http://dx.doi.org/10.1002/1097-0142(19940315)73:6%3C1625::AID-CNCR2820730614%3E3.0.CO;2-#
80. Cho E, Rosner BA, Colditz GA. Risk factors for melanoma by body site. Cancer Epidemiol Biomarkers Prev. 2005;14(5):1241–4. http://dx.doi.org/10.1158/1055-9965.EPI-04-0632
81. Elder DE. Human melanocytic neoplasms and their etiologic relationship with sunlight. J Investig Dermatol. 1989;92(5 Suppl):297s–303s. http://dx.doi.org/10.1038/jid.1989.86
82. Stierner U, Augustsson A, Rosdahl I, Suurkula M. Regional distribution of common and dysplastic naevi in relation to melanoma site and sun exposure. A case-control study. Melanoma Res. 1992;1(5–6): 367–75. http://dx.doi.org/10.1097/00008390-199201000-00008

83. Green A, MacLennan R, Youl P, Martin N. Site distribution of cutaneous melanoma in Queensland. Int J Cancer. 1993;53(2):232–6. http://dx.doi.org/10.1002/ijc.2910530210
84. Green A. A theory of site distribution of melanomas: Queensland, Australia. Cancer Causes Control. 1992;3(6):513–16. http://dx.doi.org/10.1007/BF00052747
85. Chen YT, Zheng T, Holford TR, Berwick M, Dubrow R. Malignant melanoma incidence in Connecticut (United States): Time trends and age-period-cohort modeling by anatomic site. Cancer Causes Control. 1994;5(4):341–50. http://dx.doi.org/10.1007/BF01804985
86. Lachiewicz AM, Berwick M, Wiggins CL, Thomas NE. Epidemiologic support for melanoma heterogeneity using the surveillance, epidemiology, and end results program. J Investig Dermatol. 2008;128(5):1340–2. http://dx.doi.org/10.1038/jid.2008.18
87. Stang A, Stabenow R, Eisinger B, Jockel KH. Site- and gender-specific time trend analyses of the incidence of skin melanomas in the former German Democratic Republic (GDR) including 19351 cases. Eur J Cancer (Oxford, England : 1990). 2003;39(11):1610–18. http://dx.doi.org/10.1016/S0959-8049(03)00359-9
88. Elwood JM, Gallagher RP. Body site distribution of cutaneous malignant melanoma in relationship to patterns of sun exposure. Int J Cancer. 1998;78(3):276–80. http://dx.doi.org/10.1002/(SICI)1097-0215(19981029)78:3%3C276::AID-IJC2%3E3.0.CO;2-S
89. Perez-Gomez B, Aragones N, Gustavsson P, Lope V, Lopez-Abente G, Pollan M. Do sex and site matter? Different age distribution in melanoma of the trunk among Swedish men and women. Br J Dermatol. 2008;158(4):766–72. http://dx.doi.org/10.1111/j.1365-2133.2007.08429.x
90. Dennis LK. Analysis of the melanoma epidemic, both apparent and real: Data from the 1973 through 1994 surveillance, epidemiology, and end results program registry. Arch Dermatol. 1999;135(3):275–80. http://dx.doi.org/10.1001/archderm.135.3.275
91. American Cancer Society. Cancer facts & figures 2016. Atlanta, GA: American Cancer Society; 2016 [cited 2017 Apr]. Available from: https://old.cancer.org/acs/groups/content/@research/documents/document/acspc-047079.pdf
92. Welch HG, Woloshin S, Schwartz LM. Skin biopsy rates and incidence of melanoma: Population based ecological study. BMJ (Clinical research ed). 2005;331(7515):481. http://dx.doi.org/10.1136/bmj.38516.649537.E0
93. Shaikh WR, Dusza SW, Weinstock MA, Oliveria SA, Geller AC, Halpern AC. Melanoma Thickness and Survival Trends in the United States, 1989 to 2009. J Natl Cancer Inst. 2016;108(1). pii: djv294. doi: 10.1093/jnci/djv294.
94. Ward-Peterson M, Acuna JM, Alkhalifah MK, Nasiri AM, Al-Akeel ES, Alkhaldi TM, et al. Association between race/ethnicity and survival of melanoma patients in the United States over 3 decades: A secondary analysis of SEER data. Medicine. 2016;95(17):e3315. http://dx.doi.org/10.1097/MD.0000000000003315
95. Khosrotehrani K, Dasgupta P, Byrom L, Youlden DR, Baade PD, Green AC. Melanoma survival is superior in females across all tumour stages but is influenced by age. Arch Dermatol Res. 2015;307(8):731–40. http://dx.doi.org/10.1007/s00403-015-1585-8
96. Jemal A, Devesa SS, Fears TR, Hartge P. Cancer surveillance series: Changing patterns of cutaneous malignant melanoma mortality rates among whites in the United States. J Natl Cancer Inst. 2000;92(10):811–18. http://dx.doi.org/10.1093/jnci/92.10.811
97. Stang A, Stang K, Stegmaier C, Hakulinen T, Jockel KH. Skin melanoma in Saarland: Incidence, survival and mortality 1970–1996. Eur J Cancer Prev. 2001;10(5):407–15. http://dx.doi.org/10.1097/00008469-200110000-00004
98. Balzi D, Carli P, Geddes M. Malignant melanoma in Europe: Changes in mortality rates (1970–90) in European Community countries. Cancer Causes Control. 1997;8(1):85–92. http://dx.doi.org/10.1023/A:1018491323442
99. Gaudette LA, Altmayer CA, Wysocki M, Gao RN. Cancer incidence and mortality across Canada. Health Rep. 1998;10(1):51–66(eng);55–72(fre).
100. de Vries E, Bray FI, Eggermont AM, Coebergh JW. Monitoring stage-specific trends in melanoma incidence across Europe reveals the need for more complete information on diagnostic characteristics. Eur J Cancer Prev. 2004;13(5):387–95. http://dx.doi.org/10.1097/00008469-200410000-00006
101. Chen L, Jin S. Trends in mortality rates of cutaneous melanoma in East Asian populations. PeerJ. 2016;4:e2809. http://dx.doi.org/10.7717/peerj.2809

102. Lazovich D, Choi K, Vogel RI. Time to get serious about skin cancer prevention. Cancer Epidemiol Biomarkers Prev. 2012;21(11):1893–901. http://dx.doi.org/10.1158/1055-9965.EPI-12-0327
103. Brunssen A, Waldmann A, Eisemann N, Katalinic A. Impact of skin cancer screening and secondary prevention campaigns on skin cancer incidence and mortality: A systematic review. J Am Acad Dermatol. 2017;76(1):129–39.e10. http://dx.doi.org/10.1016/j.jaad.2016.07.045
104. Nilsen LT, Aalerud TN, Hannevik M, Veierod MB. UVB and UVA irradiances from indoor tanning devices. Photochem Photobiol Sci. 2011;10(7):1129–36. http://dx.doi.org/10.1039/c1pp05029j
105. Nilsen LT, Hannevik M, Aalerud TN, Johnsen B, Friberg EG, Veierod MB. Trends in UV irradiance of tanning devices in Norway: 1983–2005. Photochem Photobiol. 2008;84(5):1100–8. http://dx.doi.org/10.1111/j.1751-1097.2008.00330.x
106. Le Clair MZ, Cockburn MG. Tanning bed use and melanoma: Establishing risk and improving prevention interventions. Prev Med Rep. 2016;3:139–44. http://dx.doi.org/10.1016/j.pmedr.2015.11.016
107. Miles A, Waller J, Hiom S, Swanston D. SunSmart? Skin cancer knowledge and preventive behaviour in a British population representative sample. Health Educ Res. 2005;20(5):579–85. http://dx.doi.org/10.1093/her/cyh010
108. Sinclair C, Foley P. Skin cancer prevention in Australia. Br J Dermatol. 2009;161(Suppl 3):116–23. http://dx.doi.org/10.1111/j.1365-2133.2009.09459.x
109. Breitbart EW, Waldmann A, Nolte S, Capellaro M, Greinert R, Volkmer B, et al. Systematic skin cancer screening in Northern Germany. J Am Acad Dermatol. 2012;66(2):201–11. http://dx.doi.org/10.1016/j.jaad.2010.11.016
110. Leiter U, Buettner PG, Eigentler TK, Garbe C. Prognostic factors of thin cutaneous melanoma: An analysis of the central malignant melanoma registry of the german dermatological society. J Clin Oncol. 2004;22(18):3660–7. http://dx.doi.org/10.1200/JCO.2004.03.074
111. Wernli KJ, Henrikson NB, Morrison CC, Nguyen M, Pocobelli G, Whitlock EP. U.S. Preventive services task force evidence syntheses, formerly systematic evidence reviews. Screening for skin cancer in adults: An updated systematic evidence review for the US preventive services task force. Rockville, MD: Agency for Healthcare Research and Quality (US); 2016.
112. Mariotto AB, Yabroff KR, Shao Y, Feuer EJ, Brown ML. Projections of the cost of cancer care in the United States: 2010–2020. J Natl Cancer Inst. 2011;103(2):117–28. http://dx.doi.org/10.1093/jnci/djq495
113. Tsao H, Rogers GS, Sober AJ. An estimate of the annual direct cost of treating cutaneous melanoma. J Am Acad Dermatol. 1998;38(5 Pt 1):669–80. http://dx.doi.org/10.1016/S0190-9622(98)70195-1

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The Epidemiology of Cutaneous Melanoma in the White and Black African Population Groups in South Africa

MARY NORVAL[1] • CARADEE Y. WRIGHT[2,3]

[1]Biomedical Sciences, University of Edinburgh Medical School, Edinburgh, United Kingdom; [2]Environment and Health Research Unit, South African Medical Research Council, Pretoria, South Africa; [3]Department of Geography, Geoinformatics and Meteorology, University of Pretoria, Pretoria, South Africa

Author for correspondence: Caradee Y. Wright, South African Medical Research Council, Private Bag x385, Pretoria, 0001, South Africa. Email: cwright@mrc.ac.za

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Abstract: In this chapter, two South African population groups, White and Black African, are compared with regard to cutaneous melanoma (CM). The incidence of CM in Black Africans is about 10% of that in Whites, explained at least in part by the protection offered by cutaneous melanin. The incidence has probably risen in Whites over the past 40 years but seems to be unchanged in Black Africans. The commonest CM subtype in Whites is superficial spreading; it occurs on various body sites, the most frequent being the trunk in males and the lower leg/hip in women. Most CMs in both male and female Black Africans are found on the lower leg and/or hip with a significant proportion being acral lentiginous melanoma, a subtype rarely seen in Whites. Risk factors including exposure to the sun,

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trauma, human immunodeficiency virus infection, albinism, age, and genetics are summarized and are likely to differ between the two population groups. The stage of CM at diagnosis tends to be more advanced in Black Africans than in Whites and, similarly, the survival rates are considerably lower in Black Africans. Reasons for the differences in CM between the two population groups are suggested.

Key words: Incidence; Mortality; Skin color; Subtypes; Sun exposure

Introduction

While surveys in several developed countries have provided accurate data on the incidence of cutaneous melanoma (CM), the resulting mortality, and the changes in these parameters over time, information from South Africa is sparse in comparison. Although reports on the epidemiology of CM in South Africa were published in the 1970s and 80s, they comprised small numbers of patients, generally attending single hospitals or clinics in one part of the country. It has remained difficult to obtain accurate figures since then, mainly due to the lack of a reliable reporting system while the country has undergone huge political, economic, and demographic changes. However, it is of considerable interest to assess what information is available as South Africa represents a subtropical country containing a multiethnic population, whose skin color ranges from deeply pigmented to fair. In this chapter, the geography of South Africa is described first, together with an explanation regarding the population groups found in this country. This section is followed by descriptions of the incidence and body sites of CM in South Africans, risk factors for CM development, the age and stage of CM at diagnosis, and mortality data. Comparisons are made throughout between the White and Black African population groups.

South Africa

GEOGRAPHY

South Africa is situated at the southern tip of Africa, spanning the midlatitudes from 22° to 34°S, and is divided into nine provinces (Figure 1). Its topography varies from coastal plains at sea level to mountain peaks reaching over 3000 m above sea level. There is a plateau at an average altitude of 1200 m, known as the Highveld, across the center of the country. High atmospheric pressure over the Highveld frequently results in relatively cloudless skies and, together with the altitude, contributes to high solar ultraviolet radiation (UVR) levels. As an illustration of the variation in the climate between the northern and southern provinces, Table 1 shows the temperature, UV Index, and the number of hours of sunshine per day in winter and summer in Cape Town (representing the South Highveld) and Pretoria (representing the North Highveld). These conditions, combined with an outdoor lifestyle, lead to the potential for excess solar UVR exposure, depending on personal phenotypic characteristics and behavior in the sun.

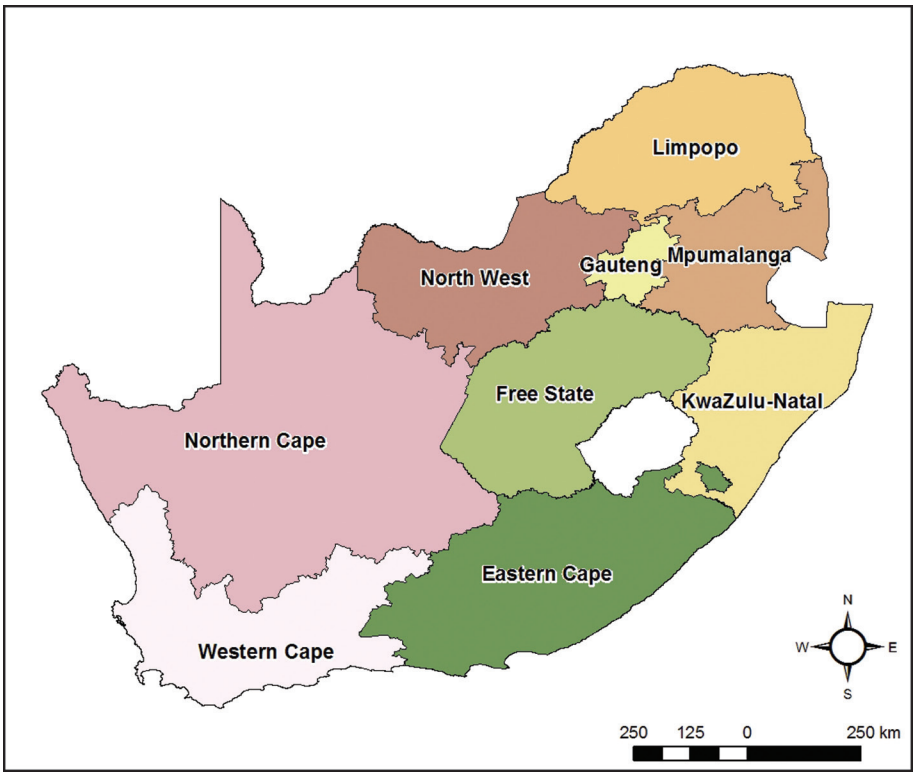

Figure 1 **The Provinces of South Africa (map drawn by M. Naidoo, Council for Scientific and Industrial Research, included with permission).**

TABLE 1 Weather Conditions in Cape Town and Pretoria (1, 2)

		Cape Town	Pretoria
	Latitude	34°S	26°S
	Altitude	0–300 m	1339 m
Average hours sunlight per day	Summer	10.2	9.1
	Winter	6.8	8.6
UV Index	Summer	9–10	11+
	Winter	2–3	4–6
Average day-time temperatures (°C)	Summer	26	30
	Winter	19	21
Average night-time temperatures (°C)	Summer	15	18
	Winter	8	5

POPULATION GROUPS

South Africa's multiethnic population comprises individuals across all six Fitzpatrick skin phototypes (3). The population is officially grouped into Black African, Colored (mixed European [White] and African ([Black] or Asian ancestry, with skin color ranging from pale to dark brown), Asian/Indian and White. The 2016 national census indicated that 80.8% were Black African, 8.6% Colored, 8.0% White, and 2.6% Indian/Asian (4). The mid-year population in 2016 was 55.9 million (4). The prevalence of several infections in South Africa is among the highest in any country in the world, particularly human immunodeficiency virus (HIV) and tuberculosis (TB). The total number of people living with HIV was estimated at approximately 7 million in 2016 (5). The incidence of TB in 2015 (including those with HIV and TB co-infections) was 834 per 100,000 people. South Africa is one of six countries that accounted for 60% of the new cases of TB in 2015 worldwide (5).

Historically, legislation restricted access for Black Africans to South African cities, but even before the repeal of the Pass Laws in 1986, the rates of rural–urban migration had increased (6). Official government projections estimate that between 2011 and 2016, the provinces of Gauteng and Western Cape experienced an inflow of 1.5 million migrants, of whom many were from the Eastern Cape and Limpopo (7). It is likely that reduced personal sun exposure, which is a recognized risk factor for some subtypes of CM, may occur with urbanization. This could be due to more time spent indoors, fewer outdoor occupations, particularly subsistence farming, and the city environment such as tall buildings and narrow streets providing more shade.

The South African National Cancer Registry (NCR) was established as a pathology-based cancer reporting system, although mandatory reporting was only legislated in 2011. Prior to this, private health laboratories withheld cancer reports from 2005 to 2007 owing to concerns regarding voluntary sharing of confidential patient data. While private health care reporting to the NCR decreased by 28% from 2005 to 2007, it is estimated that this represented a minimal impact (net decrease of <4%) on overall cancer reporting (8). The mismatch between observed and estimated number of cancers mainly impacted on affluent South Africans in all population groups who used private health laboratories since less affluent people tended to receive government-provided care.

Incidence of CM in the White and Black African Population Groups

Table 2 lists the studies that have monitored the incidence of CM in the two population groups. It is clear that the incidence is approximately 20 times higher in the White African population than in the Black African population. It is recognized that epidermal melanin provides protection against the development of skin cancer, including CM. This endogenous sun protection factor (SPF) has been estimated at up to 13.4 SPF in African Americans (17). The incidence is higher in White men than in White women, and very slightly higher in Black African women than in Black African men.

TABLE 2 **Studies in Which the Incidence of Cutaneous Melanoma (CM), Diagnosed by Histopathology, in the Black African and White Populations of South Africa has been Calculated**

Reference	Years of study	Location	Number of CM cases	Age-standardized annual incidence of CM per 100,000 persons
Isaacson (9)	1966–1975	Soweto	83	Black male, 0.72; Black female, 0.87
Saxe et al. (10)	1990–1995	Cape Town	759	White all, 24.4; White male, 27.5; White female, 22.2
Jessop et al. (11)	2001–2003	Cape Town	443	White male, 36.9; White female, 33.5
South African Melanoma Advisory Board (12)	Not stated	Cape	Not stated	All Caucasian, 69
Norval et al. (13)	2000–2004	National	3413	White male, 20.5; White female, 16.5; Black male, 1.0; Black female, 1.2
National Cancer Registry of South Africa (14)	2000	National	1506	White male, 16.7; White female, 13.1; Black male, 1.1; Black female, 1.4
National Cancer Registry of South Africa (15)	2012	National	1312	White male, 15.9; White female, 12.7; Black male, 0.8; Black female, 1.1
York et al. (16)	2008–2012	Northern Cape	135	White male, 15.8; White female, 11.8; Black male, 0.2; Black female, 0.5

The earliest study on incidence, published in 1979, was based on a small number of cases in Black Africans living in Soweto, a township close to Johannesburg with an estimated population of 1 million people (9). This was followed by two studies in Whites in Cape Town which showed an increase in incidence in both men and women when figures from 1990 to 1995 were compared with that from 2001 to 2003 (10, 11). Reported data from all of South Africa indicate that the incidence in the White population is similar to that found in European countries (18) and in Black Americans in the United States (19). Norval et al. (13) found no change in incidence in either the White or the Black African populations between 2000 and 2004, and similarly there was no increase in the national figures published by the NCR when 2000 figures (14) were compared with 2012 figures (15). It should be noted that the South African Melanoma Advisory Board in 2009 estimated the incidence of CM in Caucasians in the Cape as 69 per 100,000 (12). This figure is among the highest in the world and is

similar to current estimates in Australia (20). The basis of the Board's statement is not clear and no updates have been published since 2009.

Thus, at this point in time, there is some uncertainty about whether there might have been an upward trend in the incidence of CM in the White population in South Africa over the past 30 years, but little or no indication of an increase in Black Africans. More recent data than that of 2012 are urgently required. Second, while the risk of CM is considerably lower in Black Africans than in Whites, they make up about 80% of the population and therefore a considerable health burden is implied.

Body Sites and Subtypes of Melanoma in the White and Black African Population Groups

The percentage of CMs occurring on four body sites in the White and Black African populations in 2000–2004 in South Africa has been calculated in one study (13) and is shown in Table 3. It can be seen that there was a reasonably even distribution throughout the body in White males and females although, as also reported by Saxe et al. (10), in Whites living in Cape Town, the trunk was the predominant site in males and the lower limb and/or hip in females. The situation was markedly different in Black Africans as more than two-thirds of the CMs occurred on the lower limb and/or hip in both sexes. In confirmation, earlier surveys of Black Africans found CMs predominantly on the lower limb and sole (9), the sole and palm (21), and the foot (22). Such a distribution implies that the risk factors for CM development may differ between the White and Black African population groups in South Africa.

Superficial spreading melanoma (Figure 2) is the commonest subtype in the White population of South Africa (9, 10). Data from the NCR in 2000–2004 revealed that, when reported, the percentage of melanomas presenting as acral lentiginous melanoma (ALM) (Figure 3) was 16.6% in the Black African population compared with 0.8% in the White population (13). These figures are comparable with those reported in a US study which found that 16.7% of melanomas

TABLE 3 **The Percentage of Cutaneous Melanoma Occurring on Four Body Sites in the White and Black African Populations of South Africa, 2000–2004 (13)**

	White male	White female	Black African Male	Black African Female
Head	24.5	13.1	12.0	8.3
Trunk	37.1	22.8	12.4	6.6
Upper limb and/or shoulder	18.2	23.0	6.9	12.7
Lower limb and/or hip	20.4	41.1	68.7	72.3

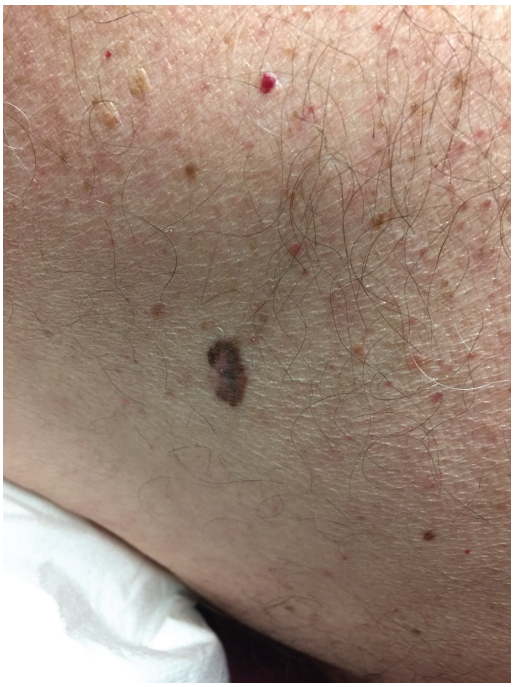

Figure 2 Superficial spreading melanoma on trunk of White male patient. Photograph supplied by Dr. W. Visser, Cape Town.

were ALM in Black Americans and only 1% of the melanomas were ALM in Whites (23). A study of 47 melanomas in Black Africans attending a referral center in Cape Town over a 14-year period (approximately 1980–1994) demonstrated that 72% were ALM, 21% were nodular, and 6% were superficial spreading (22). Hudson et al. (22) investigated plantar melanomas occurring in Black African and White patients in Cape Town between 1972 and 1985. In this period, plantar melanoma accounted for 2.1% of all the CMs in White patients, and for 73% of all CMs in Black African patients. ALM occurred in 56% of the former group but in 71% of the latter group, again demonstrating that ALM predominates in the Black African population.

Risk Factors for Melanoma in South Africa

The risk factors are complex, likely to be interrelated, and differ between population groups and anatomical site. In addition, the generation of CM is multistep, with more than one pathway being involved (reviewed in 24). One common route starts as a naevus (mole, which is a benign proliferation of melanocytes) with slow progression to melanoma *in situ*. This can then develop a vertical growth phase,

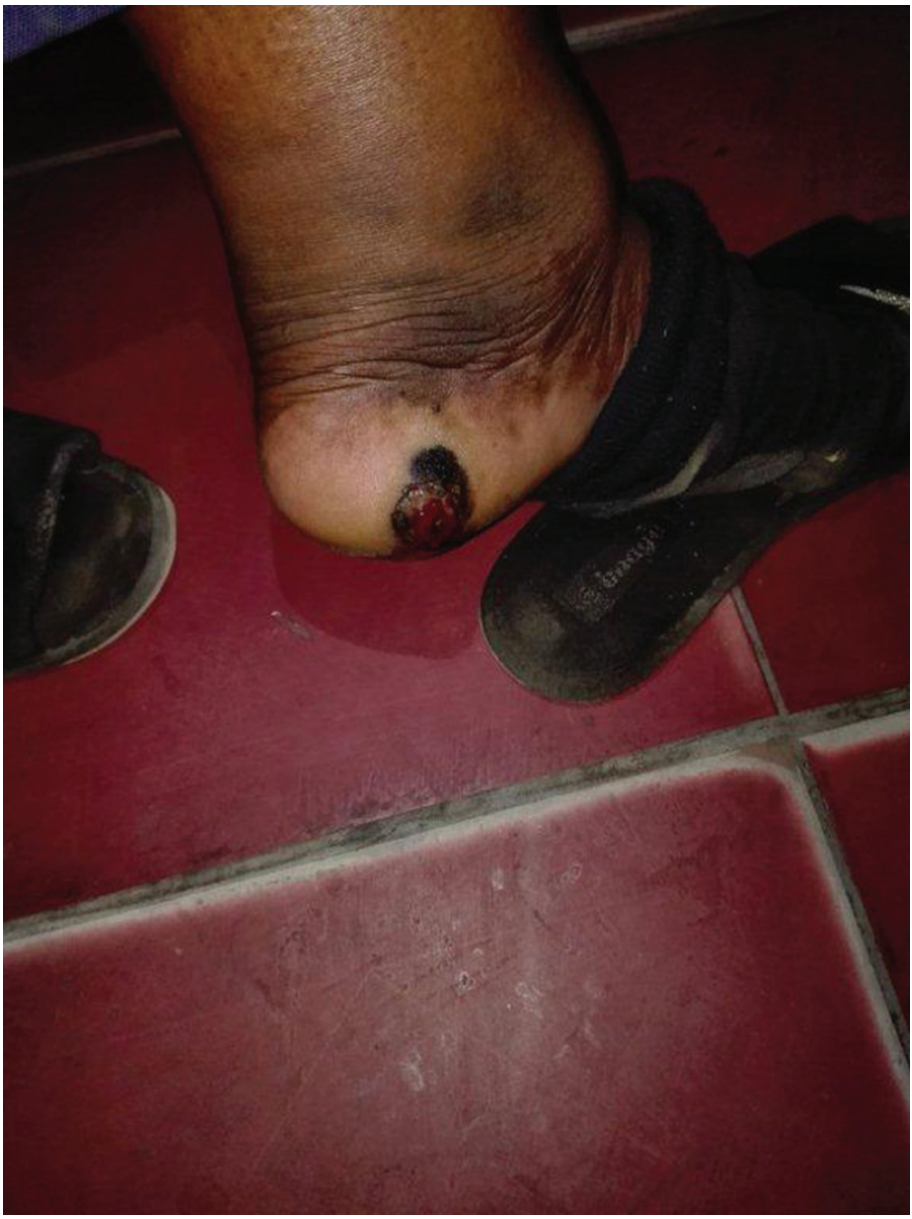

Figure 3. Acral lentiginous melanoma on the sole of a Black African female patient. Photograph supplied with permission from the patient.

invading into the dermis with the potential to metastasize. The other common route does not involve the naevi; the lesions arise spontaneously and are aggressive.

SUN EXPOSURE AND NAEVI

Solar UVR represents the major identified environmental factor with risk depending on the pattern of exposure. This differs between the subtypes of CM and has led to the hypothesis of "divergent pathways" (25). It is thought that people with few naevi tend to develop CM on body sites that are chronically exposed to the sun and show marked solar elastosis. Such sites include the face, neck, and dorsal surface of hands. Conversely, those with a high number of naevi, indicating a propensity to melanocyte proliferation, tend to develop melanomas on body sites, such as the trunk and legs. Such areas have intermittent patterns of sun exposure, including sunburn, with no solar elastosis. These pathways have been demonstrated in a large number of epidemiological and observational studies (26–28) based on Caucasians in many countries worldwide, although none have included data from South Africa. In addition, the risk of CM, as a result of sun exposure in people with hyperpigmented skin, is not clear. One analysis revealed a higher incidence of melanoma in Black Americans at lower latitudes of residence and higher mean annual UV Index in the United States, although this correlation was only significant for men (29). However, another survey of 11 cancer registries in the United States found that a higher mean UV Index was associated with an increase in melanoma incidence in Whites with some evidence for a latitude gradient in incidence. In contrast, there was no significant correlation between the UV Index and melanoma incidence in Black Americans (30). Therefore, while solar UVR is a critical risk factor in those with fair skin, it may be considerably less, or even of no importance, in those with pigmented skin.

With regard to ALM in particular, it would seem unlikely that exposure to the sun is directly involved as these tumors occur predominantly on the sole of the feet, palm of the hand, and the nail bed. However, such exposure could have systemic effects via the release of circulating immune mediators that downregulate immune responses generally (31). In addition, the pre-existing naevi on acral surfaces in deeply pigmented skin may represent a risk factor for melanoma development (32, 33). It has been reported that almost all Black people have melanocytic naevi with an average of 8.3 per person and a higher number in those with darker skin. They are predominantly acral. Approximately, one-quarter of the naevi occur on the palms and one-quarter on the soles, often near the junction between the pigmented dorsal and the nonpigmented plantar and palmer surfaces (34). As these are common sites for CM development in Black individuals, such naevi may constitute a premalignant state in those with deeply pigmented skin.

TRAUMA

As shown in Table 3, a high percentage of melanomas occur on the lower limb and/or hip in both Black African men (68.7%) and women (72.3%), and a higher incidence of ALM is found in Black Africans compared with the White population. These differences between the two population groups could be explained by

previous trauma being a risk factor in Black Africans and of less importance in Whites (32, 33). Trauma to the legs and soles could occur due to burns, scars, insect bites, and walking on bare feet.

HIV INFECTION

Currently, the estimated HIV prevalence rate in South Africa is approximately 19.2% of the total population, with a marked difference between population groups. In Black Africans, the prevalence is 15% while it is 0.3% in Whites (35). At present, almost half of those infected with HIV are being treated with antiretroviral therapy (ART) which will reduce mortality rates and may also reduce the increase in prevalence that has occurred in recent years. As persistent infection with HIV leads to a reduction in circulating CD4+ T-lymphocytes, immunodeficiency, opportunistic infections, and eventually AIDS, there is the potential for an increased risk of melanoma development in HIV-infected individuals. In addition, about 11% of HIV-infected individuals receiving ART in sub-Sahara Africa are older than 50 years, a time of increased susceptibility to a range of tumors (36). There is a wide range of HIV-associated malignancies, mostly linked with infectious agents such as Kaposi sarcoma with human herpes virus-8, Burkitt lymphoma with Epstein–Barr virus, and squamous cell carcinoma of the skin with human papillomavirus. Therefore, there is interest in determining whether HIV infection increases the risk of CM. A meta-analysis of studies performed in six countries in 2007 involving half a million patients, probably all Caucasian, indicated that the standardized incidence rate for melanoma was 1.24 per 100,000 in HIV/AIDS patients compared with uninfected controls (37). Another meta-analysis, 7 years later, compared the risk of melanoma in patients with HIV/AIDS living in North America and Europe before ART became available and after 1995 when ART was in common use (38). In about half of the included studies, an adjustment was made for ethnicity on the basis of skin color (White, Black, and other). It was found that the pooled relative risk for the association between HIV/AIDS pre-ART was 1.28 and post-ART was 1.50, when adjusted for ethnicity, compared with uninfected control groups. Thus, there is evidence that HIV infection does increase the risk of melanoma. As far as we are aware, no studies have been undertaken to monitor the relative risk of melanoma in White and Black African people with HIV/AIDS in South Africa.

SEX

A balance between the genders in Caucasians in the incidence of CM has been recorded in countries with high UV Indices, but a predominance of females over males in places with lower UV Indices (39). As seen in Table 2, the incidence of CM is higher in men than in women in the White population group in South Africa and is only slightly higher in women than in men in the Black African population group. It is possible that there are more White men than women in South Africa who have outdoor occupations, who might wear fewer clothes than women, and who use less personal photoprotection than women. Whether any gender difference in incidence or body site distribution goes beyond societal differences is uncertain at present (40, 41).

AGE

The incidence of most common cancers increases with age in both males and females worldwide. This is thought to be due to the many genetic changes involved in carcinogenesis, in addition to age-related reductions in the efficacy of the immune system. With regard to South Africa, among 595 Whites diagnosed with primary CM, the median age at diagnosis was 51 years in women and 56 years in men (10). Age-specific rates increased with age among the study sample with the steepest trends in the age group of 55 years and above. Another study reported that the mean age at presentation of melanoma among Black Africans in Cape Town was 60.5 (22), while data from the NCR indicated that the mean age in both Black African and White South Africans was approximately 55 (13). No significant difference between Black Africans and Whites in the percentage of the cases presenting under the age of 40 was demonstrated, indicating that age as a risk factor did not differ with skin color (13). In those patients living in Cape Town who developed CM on the sole of the foot or nail bed, the mean age was 56 (range 19–83) in Whites and 60.9 (range 30–83) in Black Africans (42). Therefore, there is no significant age difference between these two population groups with regard to this subtype of melanoma.

ALBINISM

Oculocutaneous albinism (OCA) is a group of congenitally inherited developmental disorders which affect the generation of pigment in the skin, iris, and hair. In South Africa, the commonest type in the Black African population is OCA2 in which the skin color is creamy white with yellow and/or light brown hair. The estimated prevalence of OCA2 in South Africa is 1 per 3900 but is considerably higher than this in some tribes (43). While people with OCA have a greatly increased susceptibility to nonmelanoma skin cancers (NMSC: squamous and basal cell carcinomas), most developing by age 20–30 years, they rarely present with CM. For example, no melanomas were found in 111 OCA patients in Johannesburg, of whom 25% had NMSC (44) and only one had an ALM in 86 OCA patients in Northern Tanzania (45). Most recently, it was reported that none of the 16 patients with OCA in Bloemfontein had a current or previous diagnosis of CM, although the majority had dendritic freckles on sun-exposed skin and were diagnosed with NMSC (46). The reason for very low frequency of melanoma in OCA may include under-reporting, especially as at least half are amelanotic (47). In addition, the life expectancy in those with OCA may not be long enough for the CM to become apparent, or the OCA2 skin color, although pale, may still offer some protection against the mutagenic effects of solar UVR in melanomagenesis.

GENETICS

Genetic susceptibility to CM is recognized and indeed a study in Sweden calculated that the familial risk for offspring of affected parents is about 2.6 times or higher if a parent has been diagnosed with CM when aged younger than 50 (48). Overall, it is estimated that 21% of the susceptibility to CM is due

to genetic factors (49). A complex range of genes is involved such as CDKN2A (a regulator of cell division) and MDm2 (a negative regulator of the p53 tumor suppressor protein). Other genes may confer a lowered risk of CM development (50). We have not found any studies comparing the genetic makeup of the White and Black African populations in South Africa, and so the contribution of inherited gene mutations in these groups as risk factors for CM is unknown at present.

Stage of Melanoma at Diagnosis in the White and Black African Population Groups

The stage of disease at diagnosis tends to be lower in Whites compared with Black Africans. Among 44 White South Africans, for example, 40 presented with Stage I melanoma of the foot, two with Stage II, one with Stage III, and one with Stage IV disease (51). An almost equal number of Black Africans were diagnosed with melanoma at Stage I (n = 30) compared with Stages II, III, and IV combined (n = 34) (22). A similar but much larger survey in the United States indicated that 16.7% of Black Americans and only 3.9% of Whites presented at Stage IV (23). Lodder et al. (52) reported that, out of 170 Black South Africans with ALM in Pretoria, 55 were Stage I, 90 were Stage II, and 25 were Stage III at the time of presentation, indicating the relatively advanced stage of disease at initial diagnosis. This point is further emphasized by 58% of the tumors being greater than 40 mm in at least one dimension on initial examination (52).

Recent evidence suggests that CM is being detected earlier, as indicated by low-stage depth increasing by 72% annually among patients in private and public health care systems in the Northern Cape Province (16). This remains to be validated in other provinces, especially given the known difficulties in early diagnosis in darkly pigmented skin types that typically lead to late presentation and the likelihood of more advanced presentation (53). Moreover, access to treatment is very poor in the public sector. This could also be related to a lack of clinical dermatology training among general practitioners. An inability to pay for appropriate treatment also contributes to more advanced stages of disease at presentation. In addition, the melanoma subtypes occurring in Black Africans may be more aggressive than in Whites, and there are likely to be significant differences in socioeconomic status and lifestyle behaviors between the two population groups.

Mortality Data for Melanoma in the White and Black African Population Groups

To date, there is little precise information on the causes of mortality in South Africa, although GLOBOCAN, which provides estimates of cancer incidence, mortality, and prevalence worldwide, reported 513 deaths due to CM in 2012 (54). One early study found that the 5-year survival rate in Black Africans with CM was 20% compared with 42% in the White population group (21). In Pretoria, 44 Black African patients out of 63 with CM died within a mean of 1 year of

presentation, while 16 were alive after a follow-up of 5 years (22). A 15-year study in Pretoria included 175 Black African patients, most of whom presented with ALM at an advanced stage (52). There were 128 documented deaths, of which 35 patients died from melanoma within 1 year of presentation. At 3 years, 92% were dead or had residual disease.

Late presentation and the malignant nature of ALM, with its propensity to metastasize, are likely to lead to poorer prognosis in Black Africans compared with White patients. Furthermore, the lack of self-examination and screening for cultural and financial reasons, plus the shortage of clinical facilities for many Black African people, particularly in rural areas, may be contributing factors.

Conclusion

The risk of developing CM is considerably less in the Black African population than in the White population in South Africa. Also, the common subtype of melanoma is superficial spreading in Whites, while ALM predominates in the Black Africans. A range of factors that may increase the risk of CM includes exposure of the skin to the sun, the presence of naevi, body sites of trauma, HIV infection, sex, age, and the occurrence of albinism and genetics. These are likely to differ markedly between the two population groups. The stage of CM at diagnosis tends to be more advanced in the Black Africans than in the Whites, with an associated reduced survival rate. Reliable published data on all these aspects are sparse and almost entirely lacking in recent years. In particular, it is important to ascertain if the incidence of CM in the White population is increasing, and whether improved public awareness about the dangers of CM is leading to earlier detection in all population groups. Finally, as Black Africans represent around 80% of the South African population currently, even the low incidence in this group implies considerable social and financial costs.

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References

1. Weather2travel. 2017. Available from: www.weather2travel.com. Accessed 24 October 2017.
2. Holiday Weather. 2017. Available at: www.holiday-weather.com. Accessed 24 October 2017.
3. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. Arch Dermatol. 1988 Jun;124(6):869–71. http://dx.doi.org/10.1001/archderm.1988.01670060015008

4. Statistics South Africa. 2017. Mid-year population estimates 2016. Available at: www.statssa.gov.za/publications/P0302/P03022016.pdf. Accessed 24 October 2017.
5. WHO. Global solar UV index: A practical guide [Internet]. 2002. Available from: http://www.who.int/uv/publications/en/GlobalUVI.pdf?ua=1. Accessed 24 October 2017.
6. Reed HE. Moving across boundaries: Migration in South Africa, 1950–2000. Demography. 2013 Feb;50(1):71–95.
7. Statistics South Africa. 2015. Mid-year population estimates 2014. Available at: www.statssa.gov.za/publications/P0302/P03022014.pdf. Accessed 24 October 2017.
8. Singh E, Underwood JM, Nattey C, Babb C, Sengayi M, Kellett P. South African National Cancer Registry: Effect of withheld data from private health systems on cancer incidence estimates. S Afr Med J. 2015 Jan;105(2):107–9. http://dx.doi.org/10.7196/SAMJ.8858
9. Isaacson C. Cancer of the skin in urban blacks of South Africa. Br J Dermatol. 1979 Mar;100(3):347–50. http://dx.doi.org/10.1111/j.1365-2133.1979.tb06210.x
10. Saxe N, Hoffman M, Krige JE, Sayed R, King HS, Hounsell K. Malignant melanoma in Cape Town, South Africa. Br J Dermatol. 1998 Jun;138(6):998–1002. http://dx.doi.org/10.1046/j.1365-2133.1998.02266.x
11. Jessop S, Stubbings H, Sayed R, Duncan-Smith J, Schneider JW, Jordaan HF. Regional clinical registry data show increased incidence of cutaneous melanoma in Cape Town. S Afr Med J. 2008 Mar;98(3):196–9.
12. South African Melanoma Advisory Board. 2017. Melanoma. Available at: www.melanoma.co.za/. Accessed 24 October 2017.
13. Norval M, Kellet P, Wright CY. The incidence and body site of skin cancers in the population groups of South Africa. Photoderm Photoimmunol Photomed. 2014 Oct;30(5):262–5. http://dx.doi.org/10.1111/phpp.12106
14. National Cancer Registry. 2000. Cancer in South Africa 2000 Full Report. Available at: www.nioh.ac.za/assets/files/2000-CancerReport-Full.pdf. Accessed 24 October 2017.
15. National Cancer Registry. 2012. Cancer in South Africa 2012 Full Report. Available at: www.nioh.ac.za/assets/files/NCR%202012%20results.pdf. Accessed 24 October 2017.
16. York K, Dlova NC, Wright CY, Khumalo NP, Kellett PE, Kassanjee R, et al. Primary cutaneous malignancies in the Northern Cape province of South Africa: A retrospective histopathological review. S Afr Med J. 2017 Jan;107(1):83–8. http://dx.doi.org/10.7196/SAMJ.2017.v107i1.10924
17. Halder RM, Bridgeman-Shah S. Skin cancer in African Americans. Cancer. 1995 Jan;75(2):s667–73. http://dx.doi.org/10.1002/1097-0142(19950115)75:2+%3C667::AID-CNCR2820751409%3E3.0.CO;2-I
18. Arnold M, Holterhues C, Hollestein LM, Coebergh JW, Nijsten T, Pukkala E, et al. Trends in incidence and predictions of cutaneous melanoma across Europe up to 2015. J Eur Acad Dermatol Venereol. 2014 Sept;28(9):1170–8. http://dx.doi.org/10.1111/jdv.12236
19. Rouhani P, Hu S, Kirsner RS. Melanoma in Hispanic and black Americans. Cancer Causes Control. 2008 Jul;15(3):248–53. http://dx.doi.org/10.1177/107327480801500308
20. Australian Government Cancer Australia. 2017. Melanoma skin cancer in Australia. Available at https://melanoma.canceraustralia.gov.au/statistics. Accessed 24 October 2017.
21. Rippey JJ, Rippey E. Epidemiology of malignant melanoma of the skin in South Africa. S Afr Med J. 1984 Apr;65(15):595–8.
22. Hudson DA, Krige JE. Melanoma in black South Africans. J Am Coll Surg. 1995 Jan;180(1):65–71.
23. Cormier JN, Xing Y, Ding M, Lee JE, Mansfield MD, Gershenwald JE, et al. Ethnic differences among patients with cutaneous melanoma. Arch Intern Med. 2006 Sept;166(17): 1907–14. http://dx.doi.org/10.1001/archinte.166.17.1907
24. Shain AH, Bastian BC. From melanocytes to melanomas. Nat Rev Cancer. 2016 Jun;16(6):345–58. http://dx.doi.org/10.1038/nrc.2016.37
25. Green A. A theory of site distribution of melanomas: Queensland, Australia. Cancer Causes Control. 1992 Nov;3(6):513–16. http://dx.doi.org/10.1007/BF00052747
26. Whiteman DC, Stickley M, Watt P, Hughes MC, Davis MB, Green AC. Anatomic site, sun exposure, and the risk of cutaneous melanoma. J Clin Oncol. 2006 Jul;24(19):3172–7. http://dx.doi.org/10.1200/JCO.2006.06.1325

27. Chang YM, Barrett JH, Armstrong BK, Bataille V, Bergman W, Berwick M, et al. Sun exposure and melanoma risk at different latitudes: A pooled analysis of 5700 cases and 7216 controls. Int J Epidemiol. 2009 Jun;38(3):814–30. http://dx.doi.org/10.1093/ije/dyp166
28. Olsen CM, Zens MS, Stukel TA, Sacerdote C, Chang YM, Armstrong BK, et al. Nevus density and melanoma risk in women: A pooled analysis to test the divergent pathway hypothesis. Int J Cancer. 2009 Feb;124(4):937–44. http://dx.doi.org/10.1002/ijc.24011
29. Hu S, Ma F, Collado-Mesa F, Kirsner RS. UV radiation, latitude, and melanoma in US Hispanics and blacks. Arch Dermatol. 2004 Jul;140(7):819–24. http://dx.doi.org/10.1001/archderm.140.7.819
30. Eide MJ, Weinstock MA. Association of UV index, latitude, melanoma incidence in nonwhite populations—US surveillance, epidemiology, and end results (SEER) program, 1992 to 2001. Arch Dermatol. 2005 Apr;141(4):477–81. http://dx.doi.org/10.1001/archderm.141.4.477
31. Agbai ON, Buster K, Sanchez M, Hernandez C, Kundu RV, Chiu M, et al. Skin cancer and photoprotection in people of color: A review and recommendations for physicians and the public. J Am Acad Dermatol. 2014 Apr;70(4):748–62. http://dx.doi.org/10.1016/j.jaad.2013.11.038
32. Rolon PA, Kramarova E, Rolon HI, Khlat M, Parkin DM. Plantar melanoma: A case-control study in Paraguay. Cancer Causes Control. 1997 Nov;8(6):850–6. http://dx.doi.org/10.1023/A:1018460227927
33. Green A, McCredie M, MacKie R, Giles G, Young P, Morton C, et al. A case-study of melanomas of the soles and palms (Australia and Scotland). Cancer Causes Control. 1999 Feb;10(1):21–5.
34. Coleman WP, Gately LE, Krementz AB, Reed RJ, Krementz ET. Nevi, lentigines, and melanomas in blacks. Arch Dermatol. 1980 May;116(5):548–51. http://dx.doi.org/10.1001/archderm.1980.01640290058011
35. Shisana O, Simbayi T, Zuma K, Jooste S, Zunga N, Labadarios D, et al. South African National HIV prevalence, incidence and behaviour survey 2012. Cape Town, South Africa: HSRC Press.
36. Cobucci RN, Lima PH, de Souza PC, Costa VV, Cornetta Mda C, Fernandes JV, et al. Assessing the impact of HAART on the incidence of defining and non-defining AIDS cancers among patients with HIV/AIDS. J Infect Public Health. 2015 Jan–Feb;8(1):1–10. http://dx.doi.org/10.1016/j.jiph.2014.08.003
37. Grulich AE, van Leeuwen MT, Falster MO, Vajdic CM. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: A meta-analysis. Lancet. 2007 Jul;370(9581):59–67. http://dx.doi.org/10.1016/S0140-6736(07)61050-2
38. Olsen CM, Knight LL, Green AC. Risk of melanoma in people with HIV/AIDS in the pre- and post-HAART eras: A systematic review and meta-analysis of cohort studies. PLoS One. 2014 Apr;9(4):e95096. http://dx.doi.org/10.1371/journal.pone.0095096
39. Buettner PG, MacLennan R. Geographical variation of incidence of cutaneous melanoma in Queensland. Aust J Rural Health. 2008 Oct;16(5):269–77. http://dx.doi.org/10.1111/j.1440-1584.2008.00987.x
40. Smith MA, Fine JA, Barnhill RL, Berwick M. Hormonal and reproductive influences and risk of melanoma in women. Int J Epidemiol. 1998 Oct;27(5):751–7. http://dx.doi.org/10.1093/ije/27.5.751
41. Koomen ER, Joosse A, Herings RM, Casparie MK, Guchelaar HJ, Nijsten T. Estrogens, oral contraceptives and hormonal replacement therapy increase the incidence of cutaneous melanoma: A population-based case-control study. Ann Oncol. 2009 Feb;20(2):358–64. http://dx.doi.org/10.1093/annonc/mdn589
42. Hudson DA, Krige JE, Stubbings H. Plantar melanoma: Results of treatment in three population groups. Surgery. 1998 Nov;124(5):877–82. http://dx.doi.org/10.1016/S0039-6060(98)70012-1
43. Lund PM, Maluleke TG, Gaigher I, Gaigher MJ. Oculocutaneous albinism in a rural community of South Africa: A population genetic study. Ann Hum Biol. 2007 Jul–Aug; 34(4):493–7. http://dx.doi.org/10.1080/03014460701401261
44. Kromberg JG, Castle D, Zwane EM, Jenkins T. Albinism and skin cancer in Southern Africa. Clin Genet. 1989 Jul;36(1):43–52. http://dx.doi.org/10.1111/j.1399-0004.1989.tb03365.x
45. Kiprono SK, Chaula BM, Beltraminelli H. Histological review of skin cancers in African albinos: A 10-year retrospective review. BMC Cancer. 2014 Mar;14:157. http://dx.doi.org/10.1186/1471-2407-14-157
46. van der Westhuizen G, Beukes CA, Green B, Sinclair W, Goedhais J. A histopathological study of melanocytic and pigmented skin lesions in patients with albinism. J Cuten Pathol. 2015 Nov;42(11):840–6. http://dx.doi.org/10.1111/cup.12588
47. Schulze KE, Rapini RP, Duvic M. Malignant melanoma in oculocutaneous albinism. Arch Dermatol. 1989 Nov;125(11):1583–6. http://dx.doi.org/10.1001/archderm.125.11.1583b

48. Hemminki K, Li X, Pina K, Granstrom C, Vaittinen P. The nation-wide Swedish family-cancer database—Updated structure and familial rates. Acta Oncol. 2001;40(6):772–7. http://dx.doi.org/10.1080/02841860152619214
49. Czene K, Lichenstein P, Hemminki K. Environmental and heritable causes of cancer among 9.6 million individuals in the Swedish family-cancer database. Int J Cancer. 2002 May;99(2):260–6. http://dx.doi.org/10.1002/ijc.10332
50. Palmer JS, Duffy DL, Box NF, Aitken JF, O'Gorman LE, Green AC, et al. Melanocortin-1 receptor polymorphisms and risk of melanoma: Is the association explained solely by pigmentation phenotype? Am J Hum Genet. 2000 Jan;66(1):176–86. http://dx.doi.org/10.1086/302711
51. Hudson DA, Fenn C, Krige JE, Johnson C. Melanoma of the foot in White South Africans. Scand J Plast Reconstr Surg Hand Surg. 1996 Dec;30(4):315–19. http://dx.doi.org/10.3109/02844319609056410
52. Lodder JV, Simson W, Becker PJ. Malignant melanoma of the skin in black South Africans: A 15-year experience. S Afr J Surg. 2010 Jul;48(3):76–9.
53. Nthumba PM, Cavadas PC, Landin L. Primary cutaneous malignancies in sub-Saharan Africa. Ann Plast Surg. 2011 Mar;66(3):313–20. http://dx.doi.org/10.1097/SAP.0b013e3181e7db9a
54. International Agency for Research on Cancer. 2017. Globocan 2012. Available at: globocan.iarc.fr. Accessed 24 October 2017.

3 Biomarkers in Malignant Melanoma: Recent Trends and Critical Perspective

BIRGIT BELTER[1] • CATHLEEN HAASE-KOHN[1] • JENS PIETZSCH[1,2]

[1]Department of Radiopharmaceutical and Chemical Biology, Institute of Radiopharmaceutical Cancer Research, Helmholtz-Zentrum Dresden-Rossendorf, Dresden, Germany; [2]Department of Chemistry and Food Chemistry, Technische Universität Dresden, Dresden, Germany

Author for correspondence: Jens Pietzsch, Department of Radiopharmaceutical and Chemical Biology, Institute of Radiopharmaceutical Cancer Research, Helmholtz-Zentrum Dresden-Rossendorf, Dresden, Germany. E-mail: j.pietzsch@hzdr.de

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Abstract: The worldwide incidence of malignant melanoma is steadily increasing, suggesting a probable melanoma "epidemic." From a clinical point of view, malignant melanoma still is an unpredictable disease and, once in the advanced stage, allows only scarce therapeutic options. There is an urgent need to identify, characterize, and validate informative biomarkers, biomarker patterns, or surrogate markers in order to not only improve early diagnosis of melanoma but also for differential diagnosis, staging, prognosis, therapy selection, and therapy monitoring. In this chapter, an update on the ongoing debate on serologic and histologic markers such as lactate dehydrogenase, tyrosinase, S100 family of calcium-binding proteins, cyclooxygenase-2, matrix metalloproteinases, and stem and/or progenitor cell markers are presented, and novel, innovative, and promising trends currently being explored are discussed.

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Key words: Cyclooxygenase-2; Lactate dehydrogenase; Malignant melanoma; Matrix metalloproteinases; S100 proteins; Tyrosinase

Introduction

Melanoma is the most common malignant type of all skin neoplasms. Although current clinical, biochemical, and histological methods provide insights into disease behavior and outcome, melanoma is still an unpredictable disease. Once metastasized, it remains a fatal neoplasm with scarce therapeutic options, despite current progress in immunomodulatory therapy. Therefore, significant efforts still need to be made in finding suitable biomarkers that could aid or improve its early diagnosis, its correct staging, the discrimination of other pathological conditions, as well as indicate patients' prognosis or the most appropriate personalized therapeutic regimes. On the other hand, well-defined diagnostic markers are strictly necessary to avoid the apparent overdiagnosis of melanoma. This chapter provides an overview of the literature on recent efforts in cutaneous malignant melanoma biomarker research. A PubMed database search was performed in March 2017 using key words and phrases such as "biomarker," "serum/plasma/tissue biomarker," "biomarker analysis," "immunohistochemistry," linked to the key words "melanoma," "malignant melanoma," and "metastatic melanoma". Regarding earlier literature, the authors refer to two very comprehensive review articles on protein and nonprotein biomarkers in melanoma published in 2012 by our group (1) and, more recently, in 2015 by Karagiannis et al. (2), with the latter, however, also mostly referring to the literature before 2013 (113 out of 130 citations).

Biomarkers in Malignant Melanoma: A Current Status

Melanoma incidence and mortality have been steadily increasing in almost all countries, especially in fair-skinned populations. Exemplarily, 2013 German incidence rates (mortality rates) of cutaneous melanoma were 19.1 (3.0) per 100,000 males and 17.4 (1.7) per 100,000 females, with cutaneous melanoma responsible for about 1.3% of all cancer deaths (Association of Population-based Cancer Registries in Germany, GEKID; http://www.gekid.de). Considering variations between countries, 5-year survival for people of all races diagnosed with primary cutaneous melanoma <1.5 mm in depth is about 90%, amounting to 99% for local disease. The 5-year survival for people diagnosed with mucosal and intraocular melanoma is about 70%. However, 5-year survival is only 60–65% if the disease is spread within the region of the primary melanoma, dramatically dropping to below 10% if widespread. Albeit screening campaigns and intensive public health programs resulting in decreasing incidence rates, especially in younger age groups, incidence and burden of melanoma continue to rise. This is mainly due to the aging population, continued high recreational sun exposure habits, changing climate

patterns, and increasing environmental contamination with carcinogenic agents (1, 3). Thus, sensitive screening, early detection of high-risk groups and personalization of therapy are the major principles of melanoma control. In this regard, biomarkers represent molecular attributes of the individual patient that will not only allow for detection and diagnosis but also answer questions about the biologic behavior of the tumor and metastases, mechanisms of resistance, and/or sensitivity to therapy. Prospectively, melanoma therapy will substantially be improved by the use of biomarkers that will (i) offer the potential to identify and treat melanoma before it is clearly visible or symptomatic, (ii) facilitate easy detection without even minimal surgical procedure, and (iii) serve as candidates for population-based screenings. In this regard, this chapter summarizes and critically discusses the current trends and perspectives in malignant melanoma biomarker research.

Melanoma biomarkers can be divided into different categories. Most of them show higher expression in melanoma cells than in normal tissue and, therefore, are used as diagnostic markers. Other biomarkers may serve as prognostic or predictive markers because of their increased expression in advanced stages of disease, as indicators of treatment response or of disease recurrence during follow-up (4). Moreover, melanoma progenitor and/or stem cell markers are of potential use for identification of cell subpopulations that exhibit critical properties like high carcinogenicity, metastatic potency, and treatment resistance. The ideal biomarker should be a metabolically and analytically stable molecule detectable and/or quantifiable in the blood or other body fluid compartments, which are accessible through minimally invasive procedures. This biomarker should allow for the diagnosis of a growing tumor in a patient or for the prediction of the likely response of a patient to a certain treatment, even earlier or better than by applying clinical imaging modalities. Thereby, the biomarker must exhibit sufficient sensitivity and specificity in order to minimize false-negative as well as false-positive results (1, 4).

At this moment, no ideal biomarker exists in the field of melanoma. Pathological characteristics of the primary melanoma, for example, tumor thickness (*Breslow index, Breslow thickness*), mitotic rate, and ulceration are important prognostic factors (5). However, these characteristics can only be determined after localization and biopsy or surgical resection of the tumor. Regarding the points mentioned above, either circulating melanoma cells or melanoma-associated extracellular molecules provide suitable noninvasive analytical access. Melanoma cells release many proteins and other molecules into the extracellular fluid. Some of these molecules can end up in the bloodstream and hence serve as potential serum biomarkers. From a pathobiochemical point of view, these biomarkers comprise molecules, including enzymes, soluble proteins and/or antigens, melanin-related metabolites, and circulating cell-free nucleic acids, released by (i) necrosis, (ii) active secretion, and (iii) ectodomain membrane shedding (1) (Table 1). These molecules exhibit different prognostic and predictive values in melanoma diagnosis, staging, and treatment monitoring (1, 4, 6, 7). On the other hand, biomarkers obtained from histological and immunohistochemical analyses of biopsy material play a very important role in melanoma management. Therefore, novel results and promising trends in this field also have been considered in this chapter.

TABLE 1 Potential Biomarkers in Malignant Melanoma

	Biomarker	Correlation with	Major laboratory methodologies	References[§]
Enzymes	LDH	prognosis, tumor stage, survival rate	photometric assay, meta-analysis[#]	(1)
	Tyrosinase	poor prognosis, survival rate, overall survival	RT-PCR, nested RT-PCR	(1)
	Cox-2	*Breslow index,* tumor progression	IHC	(1)
	MMP-1, MMP-3	disease-free survival	IHC	(1)
	MMP-9	disease, poor prognosis	ELISA	(1, 26)
	MMP-2	tumor progression	TMA, IHC	(27, 28)
	MMP-12	overall survival	IHC	(31)
	MMP-23	progression-free survival	IHC	(32)
	MT1-MMP	tumor progression	IHC	(30)
	TIMP-1	disease-free and overall survival	ELISA	(26)
	IDO		HPLC	(79)
	Cathepsin K	overall survival	IHC	(34)
	CD10	disease	cytomorphology, IHC	(33, 35)
	Legumain	overall survival		(36)
		overall survival	IHC	
Secreted proteins/ antigens	VEGF	tumor stage, survival, tumor progression	ELISA, RT-PCR	(1)
	VEGF-C, VEGFR-3	tumor burden	ELISA	(1)
	Osteopontin	*Breslow index*, survival, poor prognosis	IHC, TMA	(1)
	Galectin-3	poor prognosis, tumor progression	IHC, ELISA	(1)
	YKL-40	tumor stage, tumor progression, poor prognosis	ELISA	(1)
	MIA	survival poor prognosis	ELISA	(1)
	C-reactive protein	survival tumor progression	IP	(1)
	sICAM, sVCAM	survival	ELISA	(1)
	CEACAM	tumor stage, tumor progression, overall survival	IHC, ELISA	(1)
	CYT-MAA	tumor progression	ELISA	(1)
	MAGE	tumor progression	RT-PCR	(1)
	MART-1	tumor stage	RT-PCR	(1)
	TA90	survival, recurrence	ELISA	(1)

Table continued on following page

TABLE 1 Potential Biomarkers in Malignant Melanoma (Continued)

	Biomarker	Correlation with	Major laboratory methodologies	References§
S100 Proteins	S100B	tumor stage, survival, recurrence	ELISA, LIA	(47, 50)
	S100A2	tumor progression (negative correlation)	Northern blot	(1)
	S100A4	tumor progression	IHC	(1)
	S100A6	survival	Northern blot	(1)
	S100A8/A9	tumor progression	IHC, ELISA, FC	(67, 70)
	S100A13	tumor progression	MS, IHC	(65, 66)
	S100P	tumor progression	IHC	(1, 80)
Progenitor/ stem cell-like markers	SOX protein family	disease	IHC	(74, 75)
Metabolites*	5-S-cysteinyl-DOPA	poor prognosis, response to treatment	HPLC	(1)
	L-DOPA/L-tyrosine	tumor burden, tumor progression	HPLC	(1)
	6H5MI2C	*Breslow index*	HPLC	(1)
Nucleic acids	miRNA-221	*Breslow index*	RT-PCR	(1)
	miRNA-29c	overall survival	RT-PCR	(1)

The table was modified according to Ref. (1) (*cf. references therein*).

Biomarker abbreviations: 6H5MI2C, 6-hydroxy-5-methoxyindole-2-carboxylic acid; CEACAM, carcinoembryonic antigen-related cell adhesion molecule 1; Cox-2, cyclooxygenase-2; CYT-MAA, cytoplasmic melanoma-associated antigen; L-DOPA, L-3,4-dihydroxyphenylalanine; IDO, indoleamine-2,3-dioxygenase; LDH, lactate dehydrogenase; MAGE, melanoma-associated antigen-1; MART-1, melanoma antigen recognized by T-cells 1; MIA, melanoma inhibitory activity; MMP, matrix metalloproteinase; sICAM, soluble intercellular adhesion molecule 1; sVCAM, soluble vascular cell adhesion molecule 1; TA90, tumor-associated antigen 90; VEGF, vascular endothelial growth factor; YKL-40, heparin- and chitin-binding lectin YKL-40 (*syn.* human cartilage glycoprotein-39).

Method abbreviations: ELISA, enzyme-linked immunosorbent assay; FC, flow cytometry; HPLC, high performance liquid chromatography; IHC, immunohistochemistry; IP, immunoprecipitation; LIA, luminescence immunoassay; MS, mass spectrometry; RT-PCR, reverse transcription polymerase chain reaction; TMA, tissue microarray.

*Metabolites of melanin synthesis pathways, #meta-analysis based on AJCC melanoma staging database (5).

§The references given in the table refer to original articles, which describe novel biomarkers, and were published between 2012 and 2017. The original articles on melanoma biomarkers that have been described before 2012 were discussed in detail in Refs. (1) and (2).

LACTATE DEHYDROGENASE

Lactate dehydrogenase (LDH, EC 1.1.1.27) is a ubiquitous enzyme catalyzing the conversion of pyruvate to lactate. This reaction is essential when oxidative phosphorylation is disrupted, for instance, in anaerobic conditions and in hypoxia (8), and the latter is quite common in fast-growing tumors with high consumption of nutrients and oxygen. In the American Joint Committee on Cancer (AJCC)

staging system, serum LDH is the only serum biomarker that was accepted as a strong prognostic parameter in clinical routine for melanoma, classifying those patients with elevated serum levels in Stage IV M1C (4, 5). In the recent past, the role of LDH as a prognostic factor and as a marker for treatment response has been confirmed further. In a meta-analysis of 76 studies on the prognostic role of LDH in solid tumors, including 12 melanoma studies from 1998 to 2014, Petrelli and colleagues confirmed that high serum LDH concentration is associated with lower overall survival in melanoma patients (9). Recent studies analyzed the suitability of serum LDH as marker for outcome of advanced melanoma patients after treatment with immunomodulatory drugs. In this regard, baseline serum LDH was demonstrated to be a strong predictive factor for overall survival after ipilimumab treatment in metastatic melanoma (10). The authors further concluded that long-term benefit of ipilimumab treatment was unlikely for patients with baseline serum LDH greater than twice the upper limit of normal. An independent study showed that low baseline serum LDH is associated with favorable outcome of late-stage melanoma patients treated with ipilimumab and, therefore, confirmed that baseline serum LDH is a strong marker for prognosis in advanced melanoma (11). The suitability of serum LDH as a predictive factor was also demonstrated for therapy with further immunomodulatory drugs, anti-programmed death receptor-1 (anti-PD-1) antibodies pembrolizumab and nivolumab (12). The authors documented that anti-PD-1-treated patients with a relative reduction of serum LDH compared with their baseline LDH achieved partial remission. On the other hand, patients with an increased serum LDH level compared with the baseline LDH showed progressive disease. They conclude that serum LDH is a useful marker not only at baseline but also during treatment in patients treated with anti-PD-1 antibodies in advanced melanoma. Despite many promising results, there are also some limitations in measuring LDH as a melanoma biomarker. First of all, LDH is not an actively secreted enzyme. Thus, LDH is only released through cell damage and cell death, which occur more frequently in malignant neoplasms. However, there are also false-positive values through hemolysis; hepatocellular injuries like hepatitis, myocardial infarction, and muscle diseases; and other infectious diseases with high amounts of necrotic cells (4). Moreover, LDH is nonspecific for melanoma and elevated levels are also found in many other benign and malignant diseases.

TYROSINASE

An indicator for the presence of circulating melanoma cells and increased probability of the occurrence of metastases is the detection of tyrosinase (EC 1.14.18.1) mRNA in peripheral blood. Although the serological analyte actually is a nucleic acid isolated from circulating melanoma cells, in the literature tyrosinase often is considered as an enzyme biomarker in melanoma (1, 4). The enzyme itself is constitutively expressed in melanocytes and melanoma cells and is involved in the biosynthesis of melanin catalyzing the oxidation of L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) and of L-DOPA to DOPAquinone. Due to the fact that tyrosinase mRNA is detected through nested reverse transcription polymerase chain reaction (RT-PCR), the analytical sensitivity is very high. It is possible to detect one melanoma cell among 10^6 normal blood cells. In the last decades, however, tyrosinase mRNA expression was determined in

many different studies, resulting in a wide range of variability (30–100%). One reason might be the transient presence of tumor cells in the bloodstream. On the other hand, nonstandardized protocols for PCR-based techniques contribute to the observed variability, lower sensitivity, and different thresholds for melanoma cell detection. In order to overcome these limitations, complementary analysis of other nucleic acid–based markers should be considered. Salvianti et al. assessed the diagnostic value of a tumor-related, methylated, cell-free DNA marker, the hypermethylated *Ras association domain family 1 isoform A* promoter, in melanoma patients (13). This marker showed good predictive capability in discriminating melanoma patients (*in situ*, invasive, and metastatic) and healthy controls. Particularly, when jointly considered with circulating tumor cells analyzed both for size and tyrosinase mRNA expression, a higher sensitivity of the detection of positive cases in invasive and metastatic melanomas was obtained. Alternatively, determination of tyrosinase as a tissue biomarker also has been taken into account. In this regard, Lin et al. very recently presented a novel methodology using scanning electrochemical microscopy for mapping expression and distribution of the Type 3 copper protein tyrosinase in tissue microarrays of skin biopsies taken from melanoma patients (14). Interestingly, the progression from a homogeneous tyrosinase distribution in Stage II to a more heterogeneous pattern in Stage III was clearly visualized. Of note, the scanning electrochemical microscopy is not limited by the presence of optically interfering species, such as melanin. The authors conclude that this methodology might be implemented as a complementary prognostic technique for diagnosing metastatic and non-metastatic melanoma stages.

CYCLOOXYGENASE-2

Another enzyme marker of interest is cyclooxygenase-2, which, in theory, should be analytically accessible by measurement of certain circulating or urinary eicosanoid products of the enzyme reaction (1). Cyclooxygenase-2 is the inducible isoenzyme of cyclooxygenases (prostaglandin-H-synthases, EC 1.14.99.1) whose overexpression is implicated in a number of inflammatory or inflammation-associated processes, including tumor inflammogenesis, angiogenesis, metastasis, and radiosensitivity (15). The enzyme catalyzes the conversion of arachidonic acid into prostaglandin H_2 (PGH_2). PGH_2 afterward is converted to a multitude of eicosanoids, for example, other prostaglandins like PGE_2, prostacyclin, and thromboxanes, depending on definite downstream synthase and/or isomerase pathways present in various cell types. These eicosanoids act as potent paracrine and endocrine mediators of metabolic processes via G-protein-coupled receptors not only in homeostasis but also in inflammatory and neoplastic processes. Regarding those cyclooxygenase downstream enzymes, special attention was paid to microsomal PGE_2 synthase-1 (EC 5.3.99.3). Very recently, Kim et al. suggested a prognostic and predictive value of this enzyme in melanoma (16). However, although eicosanoid analytics made an enormous leap, particularly by progress of liquid and/or gas chromatography and mass spectrometry, a quantitative melanoma-specific profiling of plasma or urinary eicosanoids seems remote. Therefore, recent research focuses on analysis of intracellular expression of cyclooxygenase-2 in melanoma tissue specimens. In this regard, Kuźbicki et al. established an immunohistochemical scoring algorithm showing some value of

cyclooxygenase-2 as negative prognostic marker, directly correlated with other negative prognostic factors in melanoma such as tumor thickness, ulceration, and lymph node metastasis (17). In a retrospective analysis in metastatic lymph node samples obtained from melanoma patients, Panza et al. demonstrated that when cyclooxygenase-2 expression rises above a certain threshold level, it is a negative prognostic factor for human metastatic melanoma (18). They conclude that differentiation of cyclooxygenase-2 expression in more detail would help to delineate when cyclooxygenase can be defined a negative prognostic factor. Others demonstrated cyclooxygenase-2 to be a useful immunohistochemical marker for the differentiation of melanoma from benign melanocytic lesions in the oral cavity (19). Among others, these observations substantiate findings that suggest cyclooxygenase-2 expression and/or activity as both a pathogenic key player and a promising molecular target in melanoma (20). The latter, besides pharmacological targeting, offers a rationale for developing novel radiotracers for noninvasive imaging and functional characterization of cyclooxygenase-2 in melanoma. Particularly, the development of an appropriate radiotracer for positron emission tomography would provide substantial impact to the melanoma biomarker approach (21). It should be mentioned here that the development of imaging biomarkers and quantitative imaging techniques has been identified as a major and auspicious approach to move toward personalized treatment strategies. To remain with cyclooxygenase-2 as one example, quantitation of this enzyme's functional expression by imaging is assumed to be a predictive marker for radioresistance and chemoresistance and, in turn, for therapy response, particularly under hypoxic conditions (21). In the case of other target molecules, such as membrane receptors or melanin, functional imaging of molecular markers can be combined directly with targeted therapies (22).

MATRIX METALLOPROTEINASES

The human matrix metalloproteinase (MMP) family comprises 25 members in five groups: collagenases, gelatinases, stromelysins, membrane type MMPs (MT-MMP), and others. MMPs are directly implicated in almost every biological process involving matrix degradation and remodeling, for instance, in embryogenesis, normal tissue maintenance (angiogenesis, wound healing), and in pathologies such as chronic inflammatory diseases and cancer. MMPs not only degrade and process components of the ECM but also mobilize the release of growth factors from degraded matrix and cleave proteins that block growth factors (23, 24). Melanomas express a number of MMPs that are often associated with disease progression, and key roles are mostly (25) assigned to MMP-2 and MMP-9 (26). Indeed, findings differ in some ways. In a tissue microarray and immunohistochemistry study comprising 482 melanoma tumor and 149 nevi biopsies, Rotte et al. found that strong MMP-2 (EC 3.4.24.24) expression is associated with significantly poorer survival of melanoma patients but is independent of tumor thickness and ulceration (27). In contrast, Kamyab-Hesari et al. immunohistochemically analyzed 24 consecutive primary melanoma samples and found that MMP-2 expression correlates with tumor thickness in melanoma and is an independent predictive factor for lymph node involvement (28). However, in a different study, MMP-2 was found to be expressed in 96% of the analyzed uveal melanoma patients but showed no significant difference between

metastatic and nonmetastatic groups (29). In a study with patients in Stages I–III versus controls, MMP-2 expression in blood samples was similar in both groups. On the other hand, serum MMP-9 (EC 3.4.24.35) was higher in melanoma patients than in controls. However, the authors found no association between MMP-9 concentration and clinicopathological parameters, such as disease-free survival and overall survival (26). Recently, the potential of further MMPs as melanoma biomarkers and possible immunotherapeutic targets was investigated. MT1-MMP (EC 3.4.24.80; *syn.* matrix metalloproteinase-14), an activator of MMP-2, was found to be higher expressed in primary melanoma than in nevi, and its expression continues to increase during melanoma progression and portends poorer patient outcome (30). MMP-12 (EC 3.4.24.65; *syn.* macrophage metalloelastase) was also found to be increased in cutaneous melanoma compared to normal skin and was significantly associated with invasion and metastasis. Furthermore, patients with high MMP-12 level had unfavorable overall survival (31). Finally, increased MMP-23 (EC 3.4.24.-) expression in primary melanomas is inversely associated with the presence of tumor infiltrating lymphocytes, suggesting a role for tumor-derived MMP-23 in the suppression of antitumor immune responses (32).

OTHER ENZYME MARKERS

Other potential enzyme markers of melanoma currently under research, accessible mostly via immunohistochemical approaches of tissue specimens, comprise the proteases cathepsin K (EC 3.4.22.28), CD10 (EC 3.4.24.11; *syn.* neutral endopeptidase and/or neprilysin), and legumain (EC 3.4.22.34; *syn.* asparaginyl endopeptidase) (33–36). However, on the basis of only few current data, their usefulness as biomarkers still is difficult to estimate. Caution also should be considered for the enzyme aldehyde dehydrogenase 1 (ALDH-1, EC 1.2.1.'3'), which has been proposed not only as a promising therapeutic target but also as a biomarker of stem cell–like cells for certain human cancers, including melanoma (37, 38). Of interest, very recently, Taylor et al. demonstrated ALDH-1 to be an independent prognostic factor in melanoma, with results based on a score derived from immunohistochemical staining (39).

ENDOGENOUS ENZYME INHIBITORS

Tissue inhibitor of metalloproteinases (TIMPs), which are natural endogenous inhibitors of MMPs, including TIMP-1, also play a significant role in tumor development. TIMPs participate in the degradation of extracellular matrix, angiogenesis, apoptosis, differentiation, as well as in proliferation of normal and tumor cells (40). In this regard, patients with melanoma at Stages I–III in comparison with the control group had significantly higher median concentrations of serum TIMP-1, and this increase had an effect on disease-free survival and overall survival. Regarding MMP-9, the authors did not observe significant correlation between concentration of TIMP-1 and depth of invasion, clinical stage, or nodal status (26). Some attention also has been paid to other protease inhibitors, namely, maspin (serpinB5) and serpinB1, which both are members of the serine protease inhibitor superfamily. Loss of melanoma maspin has been suggested to contribute to disease progression and metastatic dissemination, but this subject is of

controversial debate (41, 42). SerpinB1 has been suggested as an indicator of chemotherapy response. Willmes et al. reported experimental and clinical data on serpinB1 expression, demonstrating that melanoma Stage IV patients showing strong serpinB1 protein expression in tumor tissue are likely to benefit from cisplatin-containing chemotherapy regimens. Moreover, serpinB1 protein expression was proved to be predictive for the outcome of cisplatin-based chemotherapy in melanoma (43).

S100 PROTEINS

The S100 family of calcium-binding proteins gained importance as both potential molecular key players and biomarkers in the etiology, progression, manifestation, and therapy of neoplastic disorders, including malignant melanoma. Twelve S100 family members are expressed in melanoma: four exhibit no change in expression (S100A8, S100A9, S100A10, and S100A11); one is downregulated (S100A2); and seven are upregulated (S100A1, S100A4, S100A6, S100A13, S100B, and S100P) (44). So far, different S100 tumor markers have been tested as prognostic factors (1, 45–47), and *in vivo* studies have confirmed that S100B, S100A4, and S100A9 contribute to melanoma progression and may be therapeutic targets (44). S100B protein is highly specific and increased levels are registered in 74–100% of patients with Stage IV melanoma (48, 49). Several studies confirmed a positive correlation between advanced stage of disease and disease-free survival (48, 50, 51). Wevers et al. showed that S100B level in Stages IIIB–IIIC patients also has a strong association with melanoma prognosis. Here, preoperative measurements of S100B and S100B measured on postoperative day 2 showed the strongest association with disease-free survival. For disease-specific survival, the preoperative S100B level seems to be the strongest independent predictor (52). S100B is further suggested to be a useful marker to monitor response to chemo- and immune-chemotherapy in metastatic malignant melanoma (53). Abusaif et al. were interested in determining whether S100B is able to monitor and predict objective tumor responses and tumor progression in vemurafenib-treated patients (54). Here, the S100B level during treatment with vemurafenib showed an initial response, but repeated measurements of S100B did not seem to be sufficient for detecting tumor progression and is thus not an alternative compared to computed tomography. Another prospective study demonstrated that S100B level during response to dabrafenib or vemurafenib treatment is of prognostic value. Here, patients with high S100B levels showed a shorter progression-free disease (55). In patients with lesions of Breslow thickness >1 mm, Swiss and German guidelines recommend S100B quantification every 3–6 months for the first 1–5 years, and every 6–12 months for years 6–10. Serum concentration appears to correlate with Breslow thickness and tumor burden measured under RECIST *(Response Evaluation Criteria In Solid Tumors)* 1.1 (8). Reports show that all Stages IIIB–IV patients with S100B higher than 0.13 µg/L had metastases, and all had distant metastases if S100B was higher than 1.6 µg/L (8). Stages of malignant melanoma and the relative hazard of death increased 5-fold when circulating S100B exceeded 0.6 µg/l (48). Only the European Society of Medical Oncology (ESMO), German and Swiss guidelines recommend serum S100B as the most accurate serologic test for follow-up having better specificity for

progressive disease versus LDH (8, 56–58). In the United States, serum S100B is not used routinely because the prognostic value is limited to advanced and/or disseminated melanoma, and LDH is the predominant serum marker (58). S100A4, also called metastasis-associated protein, is universally overexpressed in a variety of tumor entities and is an independent marker for tumor progression, invasion, metastasis, poor survival, and prognosis (1). S100A4 influences cell motility, inflammation, angiogenesis, and apoptosis due to interaction between tumor cells and their microenvironment (59–64). However, extracellular S100A4 seems to be of major importance in this context and, therefore, may possibly serve as a blood marker. Besides some initially promising results on the use of S100A4 serum levels as a prognostic marker in melanoma, the greatest problem might be that of low serum protein concentration which impedes clinical relevance (1). An attractive approach for the treatment of cancer seems to be the blocking of extracellular S100A4 with a neutralizing monoclonal antibody, leading to abolished endothelial cell migration, tumor growth, and angiogenesis *in vivo* in a melanoma subcutaneous xenograft model (60). S100A13, another promising prognostic marker for melanoma, is proposed to be an indicator of the angiogenic switch that facilitates disease progression. Massi and colleagues found expression in dysplastic nevi and in primary and metastatic melanoma with increasingly higher correlation in more aggressive and/or advanced tumors (Breslow thickness and Clark's level) (64). A proteomics study reported S100A13 to be elevated in cisplatin-resistant melanoma cell lines (65). There is also a correlation between S100A13 expression and chemotherapy resistance vis-à-vis dacarbazine and temozolomide in human melanoma tumors (66). Here, low or no expression of S100A13 could be a valuable marker to identify melanoma patients responding to chemotherapy. The calcium-binding proteins S100A8 and S100A9 can dimerize to form calprotectin, the release of which during tissue damage has been implicated in inflammation and metastasis (67). The calprotectin is one of the many proinflammatory mediators released from UVR-exposed keratinocytes. S100A8/A9 stimulates cell proliferation and migration via the pattern recognition receptor RAGE (receptor for advanced glycation end products) (68). Because of the RAGE expression in melanocytes and melanoma cells, calprotectin seems to be an activator in these cells and it is a potential target for intervention in melanomagenesis (69). The latter should also be considered regarding interaction of S100A4 with RAGE (61, 62). Another study presented evidence for S100A8/A9 as a novel predictive marker for ipilimumab treatment of metastatic Stage IV melanoma patients. A pronounced upregulation of S100A8/A9 serum levels could be detected in nonresponding patients already after the first ipilimumab infusion, and a decrease as compared with baseline levels in responding melanoma patients (70).

PROGENITOR AND/OR STEM CELL–LIKE MARKERS

Animal models have demonstrated that, aside from the aforementioned markers, other proteins can be detected in circulating melanoma cells. Some of them possibly represent melanoma progenitor and/or stem cell–like markers. This includes ATP-binding cassette (ABC) multidrug transporters and the neuroepithelial intermediate filament nestin (1, 6). In this regard, immunohistochemical analysis of

nestin performed by Akiyama et al. in various melanoma specimens revealed a positive association of nestin expression with advanced disease (71). However, in this study, compound nevi also showed high expression of nestin. Among progenitor cell markers of interest are also SOX (*Sry-related HMG-Box gene*) proteins. Some represent nuclear transcription factors in the differentiation of neural crest progenitor cells to melanocytes, while others are more versatile regulators of stem and progenitor cell fate (72, 73). The immunohistochemical profile of SOX10 was used to detect metastatic melanoma in sentinel lymph nodes with high sensitivity and specificity and is supposed to be a reliable marker for supplementing other immunohistochemical stains, like S100B or melan-A (74). On the other hand, SOX10 staining cannot discriminate melanoma metastasis from nodal nevi (74). In contrast, there is evidence that suggests that SOX2, besides nestin, can effectively differentiate nodal melanocytic nevi from metastatic melanomas and, thus, may serve as a powerful diagnostic adjunct in melanoma staging (75). The differing value of these SOX protein family members as markers well reflects the excessive heterogeneity of melanoma. The same is applicable for many other melanoma biomarkers. These results, in part, in conflicting observations, essentially complicate a final evaluation. As an example, the value of two other stem cell–like markers, CD271 (nerve growth factor receptor) and CD133 (*syn.* prominin-1), both of which have been recognized recently as crucial molecules driving melanoma initiation and metastasis, has not been clarified (76–78). Other proteins considered as melanoma biomarker candidates are given in Table 1. Furthermore, various nonprotein biomarkers are potential targets for melanoma biomarker research. Those comprise metabolites of the melanin synthesis pathways, originating from the amino acid L-tyrosine, and cell-free nucleic acids (1).

NONPROTEIN BIOMARKERS

MicroRNAs (miRNAs) are small, single-stranded noncoding RNAs that regulate gene expression in normal cellular processes, and alterations in miRNAs are involved in several pathologies such as cancer ((1, 81), *cf. references therein*). Due to their stability, detectability in serum, and easy analytical accessibility, they may also be considered as useful biomarkers in malignant melanoma (81). Alterations in the expression of miRNAs and their targets are discussed as risk factors and prognostic factors in malignant melanoma (81). In serum samples of melanoma patients, Kanemaru et al. found significantly higher miR-221 levels than in healthy controls and miR-221 levels were correlated with tumor thickness. Moreover, a longitudinal study revealed a tendency for the miR-221 levels to decrease after surgical removal of the primary tumor, and to increase again at recurrence (82). In another study, Nguyen et al. analyzed paraffin-embedded archival tissue and found miR-29c expression significantly downregulated in Stage IV melanoma compared to early-stage melanoma. Furthermore, in lymph nodes from Stage III melanoma patients, higher expression of miR-29c was found to be a significant predictor of improved overall survival (83). However, further data concerning miRNAs in patient samples are needed to better assess the potential of miRNAs as biomarkers in melanoma genesis and progression.

Conclusion

All these markers offer the potential to predict the risk of progression to metastatic disease states, treatment resistance, and disease relapse. Lack of sufficient sensitivity, specificity, and accuracy are the most relevant limitations of blood-based melanoma biomarker in clinical use. Given the heterogeneity of malignant melanoma, this is taking on a special significance. In contrast, a cluster of biomarkers for one disease would be a better diagnostic tool with much higher sensitivity, specificity, and clinical accuracy. Therefore, new investigations called "proteomic profiling" or "multimarker profiling" focus on the identification of multiple co-expressed biomarkers or signature biomarker patterns which allow early detection, staging, therapeutic monitoring, and prognostic predictions (7, 84–88). This approach can be adopted for both serum and tissue specimens. In addition, multimarker analyses of circulating tumor cells could be more useful for monitoring therapy response in melanoma patients and for providing prognostic information relating to overall survival (89, 90). Identification, establishment, and validation of the optimal combination of biomarkers for multimarker profiling is a challenge and the subject of currrent research in the melanoma field.

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References

1. Tandler N, Mosch B, Pietzsch J. Protein and non-protein biomarkers in melanoma: A critical update. Amino Acids. 2012;43:2203–30. http://dx.doi.org/10.1007/s00726-012-1409-5
2. Karagiannis P, Fittall M, Karagiannis SN. Evaluating biomarkers in melanoma. Front Oncol. 2015;4:383.
3. De Giorgi V, Gori A, Grazzini M, Rossari S, Oranges T, Longo AS, et al. Epidemiology of melanoma: Is it still epidemic? What is the role of the sun, sunbeds, Vit D, betablocks, and others? Dermatol Ther. 2012;25:392–6. http://dx.doi.org/10.1111/j.1529-8019.2012.01483.x

4. Vereecken P, Cornelis F, Van Baren N, Vandersleyen V, Baurain JF. A synopsis of serum biomarkers in cutaneous melanoma patients. Dermatol Res Pract. 2012;2012:260643. http://dx.doi.org/10.1155/2012/260643
5. Balch CM, Gershenwald JE, Soong SJ, Thompson JF, Atkins MB, Byrd DR, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol. 2009;27:6199–206. http://dx.doi.org/10.1200/JCO.2009.23.4799
6. Mimeault M, Batra SK. Novel biomarkers and therapeutic targets for optimizing the therapeutic management of melanomas. World J Clin Oncol. 2012;3:32–42. http://dx.doi.org/10.5306/wjco.v3.i3.32
7. Palmer SR, Erickson LA, Ichetovkin I, Knauer DJ, Markovic SN. Circulating serologic and molecular biomarkers in malignant melanoma. Mayo Clin Proc. 2011;86:981–90. http://dx.doi.org/10.4065/mcp.2011.0287
8. Alegre E, Sammamed M, Fernandez-Landazuri S, Zubiri L, Gonzalez A. Circulating biomarkers in malignant melanoma. Adv Clin Chem. 2015;69:47–89. http://dx.doi.org/10.1016/bs.acc.2014.12.002
9. Petrelli F, Cabiddu M, Coinu A, Borgonovo K, Ghilardi M, Lonati V, et al. Prognostic role of lactate dehydrogenase in solid tumors: A systematic review and meta-analysis of 76 studies. Acta Oncol. 2015;54:961–70. http://dx.doi.org/10.3109/0284186X.2015.1043026
10. Kelderman S, Heemskerk B, van Tinteren H, van den Brom RR, Hospers GA, van den Eertwegh AJ, et al. Lactate dehydrogenase as a selection criterion for ipilimumab treatment in metastatic melanoma. Cancer Immunol Immunother. 2014;63:449–58. http://dx.doi.org/10.1007/s00262-014-1528-9
11. Martens A, Wistuba-Hamprecht K, Geukes Foppen M, Yuan J, Postow MA, Wong P, et al. Baseline peripheral blood biomarkers associated with clinical outcome of advanced melanoma patients treated with ipilimumab. Clin Cancer Res. 2016;22:2908–18. http://dx.doi.org/10.1158/1078-0432.CCR-15-2412
12. Diem S, Kasenda B, Spain L, Martin-Liberal J, Marconcini R, Gore M, et al. Serum lactate dehydrogenase as an early marker for outcome in patients treated with anti-PD-1 therapy in metastatic melanoma. Br J Cancer. 2016;114:256–61. http://dx.doi.org/10.1038/bjc.2015.467
13. Salvianti F, Orlando C, Massi D, De Giorgi V, Grazzini M, Pazzagli M et al. Tumor-related methylated cell-free DNA and circulating tumor cells in melanoma. Front Mol Biosci. 2015;2:76.
14. Lin TE, Bondarenko A, Lesch A, Pick H, Cortes-Salazar F, Girault HH. Monitoring tyrosinase expression in non-metastatic and metastatic melanoma tissues by scanning electrochemical microscopy. Angew Chem Int Ed Engl. 2016;55:3813–16. http://dx.doi.org/10.1002/anie.201509397
15. Tondera C, Ullm S, Laube M, Meister S, Neuber C, Mosch B, et al. Optical imaging of COX-2: Studies on an autofluorescent 2,3-diaryl-substituted indole-based cyclooxygenase-2 inhibitor. Biochem Biophys Res Commun. 2015;458:40–5. http://dx.doi.org/10.1016/j.bbrc.2015.01.057
16. Kim SH, Hashimoto Y, Cho SN, Roszik J, Milton DR, Dal F, et al. Microsomal PGE2 synthase-1 regulates melanoma cell survival and associates with melanoma disease progression. Pigment Cell Melanoma Res. 2016;29:297–308. http://dx.doi.org/10.1111/pcmr.12455
17. Kuzbicki L, Lange D, Stanek-Widera A, Chwirot BW. Intratumoral expression of cyclooxygenase-2 (COX-2) is a negative prognostic marker for patients with cutaneous melanoma. Melanoma Res. 2016;26:448–56. http://dx.doi.org/10.1097/CMR.0000000000000282
18. Panza E, De Cicco P, Ercolano G, Armogida C, Scognamiglio G, Anniciello AM et al. Differential expression of cyclooxygenase-2 in metastatic melanoma affects progression free survival. Oncotarget. 2016;7:57077–85. http://dx.doi.org/10.18632/oncotarget.10976
19. de Souza do Nascimento J, Carlos R, Delgado-Azanero W, Mosqueda Taylor A, de Almeida OP, Romanach MJ, et al. Immunohistochemical expression of cyclooxygenase-2 (COX-2) in oral nevi and melanoma. J Oral Pathol Med. 2016;45:440–3. http://dx.doi.org/10.1111/jop.12385
20. Zelenay S, van der Veen AG, Bottcher JP, Snelgrove KJ, Rogers N, Acton SE et al. Cyclooxygenase-dependent tumor growth through evasion of immunity. Cell. 2015;162:1257–70. http://dx.doi.org/10.1016/j.cell.2015.08.015
21. Laube M, Kniess T, Pietzsch J. Radiolabeled COX-2 inhibitors for non-invasive visualization of COX-2 expression and activity—A critical update. Molecules. 2013;18:6311–55. http://dx.doi.org/10.3390/molecules18066311
22. Rbah-Vidal L, Vidal A, Billaud EM, Besse S, Ranchon-Cole I, Mishellany F, et al. Theranostic approach for metastatic pigmented melanoma using ICF15002, a multimodal radiotracer for both PET

imaging and targeted radionuclide therapy. Neoplasia. 2017;19:17–27. http://dx.doi.org/10.1016/j.neo.2016.11.001
23. Rodriguez D, Morrison CJ, Overall CM. Matrix metalloproteinases: What do they not do? New substrates and biological roles identified by murine models and proteomics. Biochim Biophys Acta. 2010;1803:39–54. http://dx.doi.org/10.1016/j.bbamcr.2009.09.015
24. Dye DE, Medic S, Ziman M, Coombe DR. Melanoma biomolecules: Independently identified but functionally intertwined. Front Oncol. 2013;3:252. http://dx.doi.org/10.3389/fonc.2013.00252
25. Thakur V, Bedogni B. The membrane tethered matrix metalloproteinase MT1-MMP at the forefront of melanoma cell invasion and metastasis. Pharmacol Res. 2016;111:17–22. http://dx.doi.org/10.1016/j.phrs.2016.05.019
26. Lugowska I, Kowalska M, Fuksiewicz M, Kotowicz B, Mierzejewska E, Kosela-Paterczyk H, et al. Serum markers in early-stage and locally advanced melanoma. Tumour Biol. 2015;36:8277–85. http://dx.doi.org/10.1007/s13277-015-3564-2
27. Rotte A, Martinka M, Li G. MMP2 expression is a prognostic marker for primary melanoma patients. Cell Oncol (Dordr). 2012;35:207–16. http://dx.doi.org/10.1007/s13402-012-0080-x
28. Kamyab-Hesari K, Mohtasham N, Aghazadeh N, Biglarian M, Memar B, Kadeh H. The expression of MMP-2 and Ki-67 in head and neck melanoma, and their correlation with clinic-pathologic indices. J Cancer Res Ther. 2014;10:696–700.
29. Luke J, Vukoja V, Brandenbusch T, Nassar K, Rohrbach JM, Grisanti S, et al. CD147 and matrix-metalloproteinase-2 expression in metastatic and non-metastatic uveal melanomas. BMC Ophthalmol. 2016;16:74. http://dx.doi.org/10.1186/s12886-016-0222-4
30. Shaverdashvili K, Wong P, Ma J, Zhang K, Osman I, Bedogni B. MT1-MMP modulates melanoma cell dissemination and metastasis through activation of MMP2 and RAC1. Pigment Cell Melanoma Res. 2014;27:287–96. http://dx.doi.org/10.1111/pcmr.12201
31. Zhang Z, Zhu S, Yang Y, Ma X, Guo S. Matrix metalloproteinase-12 expression is increased in cutaneous melanoma and associated with tumor aggressiveness. Tumour Biol. 2015;36:8593–600. http://dx.doi.org/10.1007/s13277-015-3622-9
32. Moogk D, da Silva IP, Ma MW, Friedman EB, de Miera EV, Darvishian F, et al. Melanoma expression of matrix metalloproteinase-23 is associated with blunted tumor immunity and poor responses to immunotherapy. J Transl Med. 2014;12:342. http://dx.doi.org/10.1186/s12967-014-0342-7
33. Long E, Ilie M, Bence C, Butori C, Selva E, Lalvee S, et al. High expression of TRF2, SOX10, and CD10 in circulating tumor microemboli detected in metastatic melanoma patients. A potential impact for the assessment of disease aggressiveness. Cancer Med. 2016;5:1022–30. http://dx.doi.org/10.1002/cam4.661
34. Rao Q, Wang Y, Xia QY, Shi SS, Shen Q, Tu P, et al. Cathepsin K in the immunohistochemical diagnosis of melanocytic lesions. Int J Clin Exp Pathol. 2014;7:1132–9.
35. Thomas-Pfaab M, Annereau JP, Munsch C, Guilbaud N, Garrido I, Paul C, et al. CD10 expression by melanoma cells is associated with aggressive behavior in vitro and predicts rapid metastatic progression in humans. J Dermatol Sci. 2013;69:105–13. http://dx.doi.org/10.1016/j.jdermsci.2012.11.003
36. Wu T, Sun L, Wu Y, Xiang R, Li Y, Rong W, et al. Prognostic value of legumain in uveal melanoma. Mol Med Rep. 2016;13:2377–84. http://dx.doi.org/10.3892/mmr.2016.4838
37. Luo Y, Dallaglio K, Chen Y, Robinson WA, Robinson SE, McCarter MD, et al. ALDH1A isozymes are markers of human melanoma stem cells and potential therapeutic targets. Stem Cells. 2012;30:2100–13. http://dx.doi.org/10.1002/stem.1193
38. Yue L, Huang ZM, Fong S, Leong S, Jakowatz JG, Charruyer-Reinwald A, et al. Targeting ALDH1 to decrease tumorigenicity, growth and metastasis of human melanoma. Melanoma Res. 2015;25:138–48. http://dx.doi.org/10.1097/CMR.0000000000000144
39. Taylor LA, Abraham RM, Tahirovic E, van Belle P, Li B, Huang L, et al. High ALDH1 expression correlates with better prognosis in tumorigenic malignant melanoma. Mod Pathol. 2017;30:634–639. http://dx.doi.org/10.1038/modpathol.2016.226
40. Ries C. Cytokine functions of TIMP-1. Cell Mol Life Sci. 2014;71:659–72. http://dx.doi.org/10.1007/s00018-013-1457-3
41. Martinoli C, Gandini S, Luise C, Mazzarol G, Confalonieri S, Giuseppe Pelicci P, et al. Maspin expression and melanoma progression: A matter of sub-cellular localization. Mod Pathol. 2014;27:412–19.

42. Ribero S, Senetta R, Osella-Abate S, Scalzo MS, Castellano I, Lentini F, et al. Prognostic role of maspin expression in melanoma: Probably far from clinical use. Histopathology. 2017;71:158–162. http://dx.doi.org/10.1111/his.13188
43. Willmes C, Kumar R, Becker JC, Fried I, Rachakonda PS, Poppe LM, et al. SERPINB1 expression is predictive for sensitivity and outcome of cisplatin-based chemotherapy in melanoma. Oncotarget. 2016;7:10117–32. http://dx.doi.org/10.18632/oncotarget.6956
44. Bresnick AR, Weber DJ, Zimmer DB. S100 proteins in cancer. Nat Rev Cancer. 2015;15:96–109. http://dx.doi.org/10.1038/nrc3893
45. Chen H, Xu C, Jin Q, Liu Z. S100 protein family in human cancer. Am J Cancer Res. 2014;4:89–115.
46. Tesarova P, Kalousova M, Zima T, Tesar V. HMGB1, S100 proteins and other RAGE ligands in cancer—Markers, mediators and putative therapeutic targets. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2016;160:1–10. http://dx.doi.org/10.5507/bp.2016.003
47. Zarogoulidis P, Tsakiridis K, Karapantzou C, Lampaki S, Kioumis I, Pitsiou G, et al. Use of proteins as biomarkers and their role in carcinogenesis. J Cancer. 2015;6:9–18. http://dx.doi.org/10.7150/jca.10560
48. Nikolin B, Djan I, Trifunovic J, Dugandzija T, Novkovic D, Djan V et al. MIA, S100 and LDH as important predictors of overall survival of patients with stage IIb and IIc melanoma. J BUON. 2016;21:691–7.
49. Weide B, Elsasser M, Buttner P, Pflugfelder A, Leiter U, Eigentler TK, et al. Serum markers lactate dehydrogenase and S100B predict independently disease outcome in melanoma patients with distant metastasis. Br J Cancer. 2012;107:422–8. http://dx.doi.org/10.1038/bjc.2012.306
50. Damude S, Hoekstra HJ, Bastiaannet E, Muller Kobold AC, Kruijff S, Wevers KP. The predictive power of serum S-100B for non-sentinel node positivity in melanoma patients. Eur J Surg Oncol. 2016;42:545–51. http://dx.doi.org/10.1016/j.ejso.2015.12.010
51. Kruijff S, Hoekstra HJ. The current status of S-100B as a biomarker in melanoma. Eur J Surg Oncol. 2012;38:281–5. http://dx.doi.org/10.1016/j.ejso.2011.12.005
52. Wevers KP, Kruijff S, Speijers MJ, Bastiaannet E, Muller Kobold AC, Hoekstra HJ. S-100B: A stronger prognostic biomarker than LDH in stage IIIB-C melanoma. Ann Surg Oncol. 2013;20:2772–9. http://dx.doi.org/10.1245/s10434-013-2949-y
53. Felix J, Cassinat B, Porcher R, Schlageter MH, Maubec E, Pages C, et al. Relevance of serum biomarkers associated with melanoma during follow-up of anti-CTLA-4 immunotherapy. Int Immunopharmacol. 2016;40:466–73. http://dx.doi.org/10.1016/j.intimp.2016.09.030
54. Abusaif S, Jradi Z, Held L, Pflugfelder A, Weide B, Meier F, et al. S100B and lactate dehydrogenase as response and progression markers during treatment with vemurafenib in patients with advanced melanoma. Melanoma Res. 2013;23:396–401. http://dx.doi.org/10.1097/CMR.0b013e3283650741
55. Sanmamed MF, Fernandez-Landazuri S, Rodriguez C, Lozano MD, Echeveste JI, Perez Gracia JL, et al. Relevance of MIA and S100 serum tumor markers to monitor BRAF inhibitor therapy in metastatic melanoma patients. Clin Chim Acta. 2014;429:168–74. http://dx.doi.org/10.1016/j.cca.2013.11.034
56. Dummer R, Hauschild A, Guggenheim M, Keilholz U, Pentheroudakis G, ESMO Guidelines Working Group. Cutaneous melanoma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2012;23(Suppl 7):vii86–91. http://dx.doi.org/10.1093/annonc/mds229
57. Garbe C, Peris K, Hauschild A, Saiag P, Middleton M, Bastholt L, et al. Diagnosis and treatment of melanoma. European consensus-based interdisciplinary guideline—Update 2016. Eur J Cancer. 2016;63:201–17. http://dx.doi.org/10.1016/j.ejca.2016.05.005
58. Trotter SC, Sroa N, Winkelmann RR, Olencki T, Bechtel M. A global review of melanoma follow-up guidelines. J Clin Aesthet Dermatol. 2013;6:18–26.
59. Haase-Kohn C, Wolf S, Herwig N, Mosch B, Pietzsch J. Metastatic potential of B16-F10 melanoma cells is enhanced by extracellular S100A4 derived from RAW264.7 macrophages. Biochem Biophys Res Commun. 2014;446:143–8. http://dx.doi.org/10.1016/j.bbrc.2014.02.126
60. Hernandez JL, Padilla L, Dakhel S, Coll T, Hervas R, Adan J, et al. Therapeutic targeting of tumor growth and angiogenesis with a novel anti-S100A4 monoclonal antibody. PLoS One. 2013;8:e72480. http://dx.doi.org/10.1371/journal.pone.0072480
61. Herwig N, Belter B, Pietzsch J. Extracellular S100A4 affects endothelial cell integrity and stimulates transmigration of A375 melanoma cells. Biochem Biophys Res Commun. 2016;477:963–9. http://dx.doi.org/10.1016/j.bbrc.2016.07.009

62. Herwig N, Belter B, Wolf S, Haase-Kohn C, Pietzsch J. Interaction of extracellular S100A4 with RAGE prompts prometastatic activation of A375 melanoma cells. J Cell Mol Med. 2016;20:825–35. http://dx.doi.org/10.1111/jcmm.12808
63. Kircher DA, Silvis MR, Cho JH, Holmen SL. Melanoma brain metastasis: Mechanisms, models, and medicine. Int J Mol Sci. 2016;17:pii:E1468. http://dx.doi.org/10.3390/ijms17091468
64. Massi D, Landriscina M, Piscazzi A, Cosci E, Kirov A, Paglierani M, et al. S100A13 is a new angiogenic marker in human melanoma. Mod Pathol. 2010;23:804–13. http://dx.doi.org/10.1038/modpathol.2010.54
65. Paulitschke V, Haudek-Prinz V, Griss J, Berger W, Mohr T, Pehamberger H, et al. Functional classification of cellular proteome profiles support the identification of drug resistance signatures in melanoma cells. J Proteome Res. 2013;12:3264–76. http://dx.doi.org/10.1021/pr400124w
66. Azimi A, Pernemalm M, Frostvik Stolt M, Hansson J, Lehtio J, Egyhazi Brage S, et al. Proteomics analysis of melanoma metastases: Association between S100A13 expression and chemotherapy resistance. Br J Cancer. 2014;110:2489–95. http://dx.doi.org/10.1038/bjc.2014.169
67. Hibino T, Sakaguchi M, Miyamoto S, Yamamoto M, Motoyama A, Hosoi J, et al. S100A9 is a novel ligand of EMMPRIN that promotes melanoma metastasis. Cancer Res. 2013;73:172–83. http://dx.doi.org/10.1158/0008-5472.CAN-11-3843
68. Meghnani V, Wagh A, Indurthi VS, Koladia M, Vetter SW, Law B, et al. The receptor for advanced glycation end products influences the expression of its S100 protein ligands in melanoma tumors. Int J Biochem Cell Biol. 2014;57:54–62. http://dx.doi.org/10.1016/j.biocel.2014.10.001
69. Shirley SH, von Maltzan K, Robbins PO, Kusewitt DF. Melanocyte and melanoma cell activation by calprotectin. J Skin Cancer. 2014;2014:846249. http://dx.doi.org/10.1155/2014/846249
70. Gebhardt C, Sevko A, Jiang H, Lichtenberger R, Reith M, Tarnanidis K, et al. Myeloid cells and related chronic inflammatory factors as novel predictive markers in melanoma treatment with ipilimumab. Clin Cancer Res. 2015;21:5453–9. http://dx.doi.org/10.1158/1078-0432.CCR-15-0676
71. Akiyama M, Matsuda Y, Ishiwata T, Naito Z, Kawana S. Nestin is highly expressed in advanced-stage melanomas and neurotized nevi. Oncol Rep. 2013;29:1595–9. http://dx.doi.org/10.3892/or.2013.2287
72. Hong CS, Saint-Jeannet JP. Sox proteins and neural crest development. Semin Cell Dev Biol. 2005;16:694–703. http://dx.doi.org/10.1016/j.semcdb.2005.06.005
73. Kamachi Y, Kondoh H. Sox proteins: Regulators of cell fate specification and differentiation. Development. 2013;140:4129–44. http://dx.doi.org/10.1242/dev.091793
74. Willis BC, Johnson G, Wang J, Cohen C. SOX10: A useful marker for identifying metastatic melanoma in sentinel lymph nodes. Appl Immunohistochem Mol Morphol. 2015;23:109–12. http://dx.doi.org/10.1097/PAI.0000000000000097
75. Chen PL, Chen WS, Li J, Lind AC, Lu D. Diagnostic utility of neural stem and progenitor cell markers nestin and SOX2 in distinguishing nodal melanocytic nevi from metastatic melanomas. Mod Pathol. 2013;26:44–53. http://dx.doi.org/10.1038/modpathol.2012.132
76. Ballotti R. Identification of melanoma initiating cells: Does CD271 have a future? Future Oncol. 2015;11:1587–90. http://dx.doi.org/10.2217/fon.15.24
77. Cheli Y, Bonnazi VF, Jacquel A, Allegra M, De Donatis GM, Bahadoran P, et al. CD271 is an imperfect marker for melanoma initiating cells. Oncotarget. 2014;5:5272–83. http://dx.doi.org/10.18632/oncotarget.1967
78. Madjd Z, Erfani E, Gheytanchi E, Moradi-Lakeh M, Shariftabrizi A, Asadi-Lari M. Expression of CD133 cancer stem cell marker in melanoma: A systematic review and meta-analysis. Int J Biol Markers. 2016;31:e118–25. http://dx.doi.org/10.5301/jbm.5000209
79. de Lecea MV, Palomares T, Al Kassam D, Cavia M, Geh JLC, de Llano P, et al. Indoleamine 2,3 dioxygenase as a prognostic and follow-up marker in melanoma. A comparative study with LDH and S100B. J Eur Acad Dermatol Venereol. 2017 31:636–42. http://dx.doi.org/10.1111/jdv.13968
80. Zhu L, Ito T, Nakahara T, Nagae K, Fuyuno Y, Nakao M, et al. Upregulation of S100P, receptor for advanced glycation end products and ezrin in malignant melanoma. J Dermatol. 2013;40:973–9. http://dx.doi.org/10.1111/1346-8138.12323
81. Varamo C, Occelli M, Vivenza D, Merlano M, Lo Nigro C. MicroRNAs role as potential biomarkers and key regulators in melanoma. Genes Chromosomes Cancer. 2017;56:3–10. http://dx.doi.org/10.1002/gcc.22402

82. Kanemaru H, Fukushima S, Yamashita J, Honda N, Oyama R, Kakimoto A, et al. The circulating microRNA-221 level in patients with malignant melanoma as a new tumor marker. J Dermatol Sci. 2011;61:187–93. http://dx.doi.org/10.1016/j.jdermsci.2010.12.010
83. Nguyen T, Kuo C, Nicholl MB, Sim MS, Turner RR, Morton DL, et al. Downregulation of microRNA-29c is associated with hypermethylation of tumor-related genes and disease outcome in cutaneous melanoma. Epigenetics. 2011;6:388–94. http://dx.doi.org/10.4161/epi.6.3.14056
84. Angi M, Kalirai H, Prendergast S, Simpson D, Hammond DE, Madigan MC et al. In-depth proteomic profiling of the uveal melanoma secretome. Oncotarget. 2016;7:49623–35. http://dx.doi.org/10.18632/oncotarget.10418
85. Dowling P, Moran B, McAuley E, Meleady P, Henry M, Clynes M, et al. Quantitative label-free mass spectrometry analysis of formalin-fixed, paraffin-embedded tissue representing the invasive cutaneous malignant melanoma proteome. Oncol Lett. 2016;12:3296–304. http://dx.doi.org/10.3892/ol.2016.5101
86. Pham TV, Piersma SR, Oudgenoeg G, Jimenez CR. Label-free mass spectrometry-based proteomics for biomarker discovery and validation. Expert Rev Mol Diagn. 2012;12:343–59. http://dx.doi.org/10.1586/erm.12.31
87. Romaine ST, Wells-Jordan P, de Haro T, Dave-Thakrar A, North J, Pringle JH, et al. A small multimarker panel using simple immunohistochemistry methods is an adjunct to stage for cutaneous melanoma prognosis. Melanoma Res. 2016;26:580–7. http://dx.doi.org/10.1097/CMR.0000000000000293
88. Solassol J, Du-Thanh A, Maudelonde T, Guillot B. Serum proteomic profiling reveals potential biomarkers for cutaneous malignant melanoma. Int J Biol Markers. 2011;26:82–7. http://dx.doi.org/10.5301/JBM.2011.8344
89. Arenberger P, Fialova A, Gkalpakiotis S, Pavlikova A, Puzanov I, Arenbergerova M. Melanoma antigens are biomarkers for ipilimumab response. J Eur Acad Dermatol Venereol. 2017;31:252–9. http://dx.doi.org/10.1111/jdv.13940
90. Klinac D, Gray ES, Freeman JB, Reid A, Bowyer S, Millward M, et al. Monitoring changes in circulating tumour cells as a prognostic indicator of overall survival and treatment response in patients with metastatic melanoma. BMC Cancer. 2014;14:423. http://dx.doi.org/10.1186/1471-2407-14-423

4 Heterogeneity and Plasticity of Melanoma: Challenges of Current Therapies

MARY J. C. HENDRIX[1,2,3] • ELISABETH A. SEFTOR[2,3,4] • NAIRA V. MARGARYAN[2,3,4] • RICHARD E. B. SEFTOR[2,3,4]

[1]Department of Internal Medicine, West Virginia University, Morgantown, WV, USA; [2]Cancer Institute, West Virginia University, Morgantown, WV, USA; [3]Department of Biology, Shepherd University, Shepherdstown, WV, USA; [4]Department of Biochemistry, Robert C. Byrd Health Sciences Center, West Virginia University, Morgantown, WV, USA

Author for correspondence: Mary J. C. Hendrix, Department of Internal Medicine and Cancer Institute, West Virginia University, Morgantown, WV 26506, USA; Department of Biology, Shepherd University, Shepherdstown, WV 25443, USA. E-mail: mhendrix@shepherd.edu

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Abstract: The heterogeneity and plasticity of aggressive melanoma presents formidable challenges in the design of current therapies. Plasticity is defined as the phenotype of cancer cells expressing properties normally related to stem cells, including the expression of genes associated with multiple cellular phenotypes and appearing as undifferentiated, embryonic-like cells. The multipotent phenotype of these tumor cells, expressing vascular, embryonic, and cancer stem cell (CSC) capabilities, offers new insights into their functional adaptation and resistance to current therapies. This chapter highlights major advances in research that (i) help clarify the underlying challenges associated with angiogenesis inhibitor therapy; (ii) discuss important implications of the discovery of reactivation of the normally dormant Nodal embryonic signaling pathway that underlies the CSC phenotype, unregulated tumor growth and metastasis, and resistance to current

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therapies; and (iii) demonstrate the advantage of using combinatorial strategies to effectively target heterogeneous melanoma subpopulations to eliminate relapse and disease progression.

Key words: Chemoresistance; Heterogeneity; Melanoma; Nodal; Plasticity

Introduction

Tumor heterogeneity presents a significant conundrum pertinent to the design of effective therapeutic approaches that mitigate residual disease and progression to metastasis. The complexity of this issue has not been fully appreciated until the dawn of genomic analysis and the revelation of various subpopulations of tumor cells within a tumor lesion expressing multiple phenotype-specific genes and diverse protein markers, especially prevalent in aggressive melanoma (1). At first, these findings seemed enigmatic; however, they prompted further experimental studies into the biological and clinical relevance of a multi-potent or plastic tumor cell phenotype. Most noteworthy, patients with metastatic disease were relapsing following conventional therapies, which strongly suggested the critical need for a refocused approach utilizing targeted therapies.

After years of clinical trials, preventive sunscreen advocacy, and personalized targeted therapies, metastatic melanoma remains the most aggressive and deadly type of skin cancer. In advanced-state metastatic disease, the latest statistics reveal a median overall survival of less than 6 months (2). FDA-approved agents have included a spectrum of products ranging from conventional chemotherapy such as dacarbazine (DTIC) (3), to ipilimumab—a monoclonal antibody that targets the regulatory checkpoint CTLA-4 in T-cells (4), in addition to inhibitors of mutationally activated BRAF (BRAFi) (5, 6), which have been used in combination with trametinib, an inhibitor of the mitogen-activated, extracellular signal–regulated kinase inhibitor (MEK) (7). Additional therapeutic approaches have recently included agents that target the programmed death 1 pathway (8). Despite these noteworthy advances in treatment strategies, an urgent clinical need remains to achieve improved progression-free and overall survival. However, one of the most difficult challenges to address is cellular heterogeneity within aggressive tumors, as depicted in Figure 1. First-line therapies can target portions of a primary tumor, but residual disease can arise from subpopulations of cancer cells with stem cell properties. Metastatic disease can arise from the expansion of cancer stem cells (CSCs) with drug resistance properties, which are not targeted by current therapies. Acquiring a better understanding of the molecular underpinnings of the subpopulations that express a plastic phenotype—and may appear as vascular and embryonic in nature—will lead to the development of new cancer interventions.

Melanoma Vascular Phenotype

The pioneering work of Dr Judah Folkman with respect to tumor angiogenesis initiated a critical paradigm for strategically targeting the blood supply to tumors, and guided the pharmaceutical industry to develop antiangiogenesis agents with

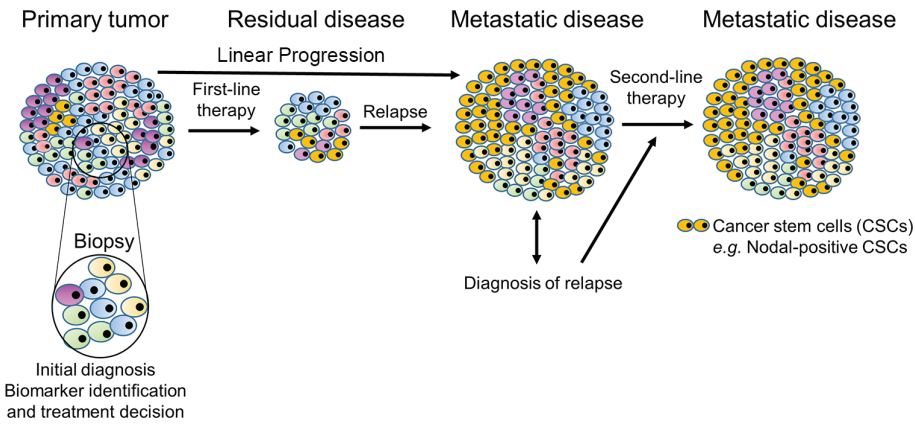

Figure 1 Tumors are comprised of heterogeneous subpopulations of melanoma cells. Generally, a diagnosis from a primary tumor biopsy is based on a "snapshot" of the cellular makeup of a small portion of the tumor mass. Analysis of the cellular composition of the biopsy reveals biomarkers which inform the type of the best front-line therapies suited for treating the tumor. With reduction in the mass of the tumor, cells unaffected by the initial treatment remain and can lead to a relapse of the tumor. Additional biopsies can then lead to second-line treatment regimes. Of note, CSCs, such as those expressing the embryonic morphogen Nodal, that are present in the primary tumor can expand and demonstrate multidrug resistance and lead to linear progression and relapse of the tumor.

the goal of inhibiting growth through nutrient starvation (9). However, as disappointment grew over the outcomes of angiogenesis inhibitor clinical trials, researchers took a closer look at the molecular signature of tumor cells that appeared resistant to this new class of agents. In the case of melanoma, there was confounding molecular evidence, indicating that aggressive melanoma cells express multiple cellular phenotypes, including those closely associated with endothelial cells, epithelial cells, and stem cells—suggesting an unusual plasticity with uncertain significance (1, 10). From a purely scientific perspective, these results were fascinating at the time but fostered serious questions and concerns about cell-type-specific markers that were used to characterize tumor cells versus normal cells. Essentially, depending on the marker selected, melanoma cells could masquerade as endothelial cells because both cell types express endothelial-specific proteins. Most noteworthy, during histopathology examination, tumor cells could be underestimated or at worst go undetected.

When the functional relevance of vascular markers was tested in melanoma models, this resulted in the surprising observation that aggressive melanoma cells expressing endothelial markers can form *de novo*, perfusable, vasculogenic-like networks in three-dimensional culture (3-D), which we named vasculogenic or vascular mimicry (11, 12). Ultrastructural analysis of these networks revealed a remarkable similarity between tumor cell–formed vessels versus endothelial lined vessels, with the exception of the basement membrane lining (13). In tumor cell–formed vessels, blood passes through basement membrane–lined vascular networks with tumor cells sitting exterior to the membrane matrix, while traditional

vessels support blood flowing through endothelial cells lining the vasculature with the basement membrane exterior to the cells. The light microscopic morphological characterization of VM in patient tumors showed matrix-rich channels containing plasma and RBCs lined by melanoma cells, and noteworthy poor clinical outcome in patients where VM was identified (14). Because VM is associated with the aggressive tumor cell phenotype and advanced stage disease, it is hypothesized that this extravascular perfusion pathway serves as a growth advantage and escape route for rapidly growing tumor cells.

When the concept of VM was first presented, it was considered quite controversial (15, 16). However, with the persistent lack of success of angiogenesis inhibitors, the VM paradigm received a serious, second look. Particularly noteworthy was the critical experiment conducted by our laboratory and collaborators, which consisted of a side-by-side comparative analysis of the effects of endostatin (a classical angiogenesis inhibitor) on endothelial cell formation of angiogenic networks versus melanoma cell–formed VM networks (17). In this straightforward experiment using 3-D cultures, the data revealed the inhibitory effect of endostatin on angiogenesis as expected, but melanoma VM was unaffected. This observation prompted further assessment of the underlying molecular mechanisms that might help explain the noteworthy differential response. We chose to specifically measure the integrin α_5-subunit (the endostatin target) expression in human endothelial cells and human metastatic melanoma cells, and found a high level of integrin α_5-subunit expressed (at the gene and protein levels) by endothelial cells and little to no expression of this endostatin target by melanoma tumor cells.

These results provided a substantial explanation for the failure of angiogenesis inhibitors in targeting tumors containing VM pathways, especially prominent in aggressive disease states. Shortly after this revelation, VM was officially adopted as one of the vascular supply routes contributing to the tumor vasculature (18), which would eventually prompt the design of more rational, targeted vascular disrupting agents. This strategic approach was further informed by microgenomics studies conducted by our laboratory consisting of a comparative molecular analysis of laser capture microdissected networks formed during angiogenesis versus melanoma VM (19). These findings revealed factors contributing to tumor plasticity, in addition to documenting important differences and similarities in angiogenesis compared with VM, especially the heterogeneous subpopulations engaged in various aspects of VM. Most noteworthy, new targets for vascular disruption were discovered in this study, which supported the development for a new class of agents.

Melanoma Embryonic Phenotype

The microgenomics study contributed valuable insights into the key players responsible for VM functionality and also introduced a new avenue of investigation in our laboratory focused on understanding the implications of the embryonic phenotype of melanoma, which feature prominently in sustaining plasticity. This direction was also supported by developmental biology findings

showing that cytotrophoblasts engage in VM during the formation of the placenta (20), and accentuated the notion that tumors can recapitulate early developmental events. To gain a broader perspective of the melanoma embryonic phenotype, a molecular comparative analysis was performed on human embryonic stem cells (hESCs) and human melanoma cells expressing the aggressive, multipotent phenotype. These studies revealed the robust expression of a Nodal embryonic signaling pathway in melanoma cells, which was present in the aggressive phenotype but not in the nonaggressive phenotype (21, 22).

Since this was the first description of Nodal in cancer, we searched the literature for information pertaining to its possible function and found the primary resource to be developmental studies (23). Nodal is a powerful embryonic morphogen belonging to the TGF-beta superfamily. It is critical in the maintenance of hESC pluripotency, as well as axis formation and L-R patterning. Nodal can act in an autocrine and paracrine manner, and is largely restricted to embryonic tissues and mostly lost in normal tissues. While hESCs and aggressive, multipotent melanoma cells share Nodal expression in common, only hESCs express the natural inhibitor of Nodal—called Lefty, also a member of the TGF-beta superfamily. Our findings revealed that while Nodal is reactivated in aggressive tumor cells, Lefty is mostly silenced through methylation (24). These observations gave us additional clues relevant to aggressive melanoma cells and the underlying embryonic phenotype. We postulated, and then confirmed, that Nodal expression contributes to the growth of melanoma tumors, and this embryonic signaling pathway is unregulated due to the absence of Lefty, allowing uncontrolled proliferation (22). We also hypothesized that Nodal is a master plasticity gene, based on its quintessential role in hESCs, and we tested this theory by downregulating Nodal expression in melanoma cells and observed a direct impact on phenotype. Specifically, when the melanoma cells no longer expressed Nodal, they acquired a more normal melanocytic phenotype, downregulated their vascular phenotype, were unable to engage in VM, and had a diminished capacity to form tumors (21).

The translational relevance of the Nodal finding was further validated by our laboratory and others using patient tissues and immunohistochemistry (IHC) analyses. Nodal was found to be associated with advanced stages of melanoma, breast, prostate, pancreatic, ovarian and colon cancer, in addition to glioblastoma and neuroblastoma (25). Collectively, these results supported the potential of Nodal as a valuable prognostic biomarker and promising new target to inhibit tumorigenicity and metastasis (26). To pursue this concept, we tested the effects of anti-Nodal antibody therapy on melanoma mouse models injected with metastatic tumor cells. The results showed a reduction in tumor growth at the primary site of orthotopic injection, and a reduction in lung tumor burden in the experimental metastasis model (25, 27, 28). Although these studies were promising, this approach using monotherapy to target only Nodal-expressing melanoma cells did not completely inhibit tumor formation. These data, together with FACS analyses revealing only a minor percentage of melanoma cells actually express Nodal, persuaded us to reevaluate our approach to effectively target aggressive melanoma, which led us to more carefully consider the CSC phenotype in subpopulations of melanoma.

Cancer Stem Cell Phenotype

Guided by the implications of CSCs, as illustrated in Figure 1, expanding their influence during tumor progression because they are able to survive current therapies, we hypothesized that the melanoma cells expressing Nodal would also express a well-characterized CSC marker, CD133, also associated with drug resistance (29). We employed SmartFlare™ technology to selectively sort and study the functional relevance of Nodal subpopulations existing within heterogeneous melanoma cell lines (30). The results indicated that melanoma subpopulations selected for Nodal expression concomitantly expressed CD133 and displayed significant tumorigenic growth in soft agar compared with nonselected cells.

These experiments stimulated a line of inquiry specifically focused on the question of whether current therapies for metastatic melanoma patients were targeting Nodal. Starting with dacarbazine (DTIC), FDA approved in the 1970s, we discovered that the residual tumor cells surviving treatment are strongly Nodal-positive (31). However, a combinatorial approach of treating with DTIC followed by anti-Nodal antibody treatment was most effective in causing cell death, accompanied by the expression of cleaved PARP (an apoptosis marker). Further support for the critical need of new therapeutic approaches, also revealed in this study, showed prominent IHC Nodal localization in patient tissues before and after DTIC treatment. Despite DTIC failing for most patients, it is still used as the front-line therapy in many cases.

Melanoma patients, like many others with cancer, could benefit from targeted therapies as part of the era of personalized medicine. However, despite advances in the field, the heterogeneity of melanoma—especially the CSC subpopulations expressing Nodal and drug resistance markers—complicate our ability to mitigate relapse and progression to metastasis with current therapeutic options. To address this clinical challenge, our laboratory and collaborators examined whether melanoma patients treated with BRAFi therapy experienced a change in the Nodal-expressing tumor cells. The results showed that BRAFi treatment failed to affect Nodal levels in matched melanoma patient samples before and after therapy—that preceded their eventual death due to disease (32). These data encouraged us to perform an experimental assessment using a mouse model with tagged human metastatic melanoma cells, comparing groups treated with monotherapies of BRAFi or anti-Nodal mAb or a combination of both versus controls. The results clearly demonstrated the efficacy of using the combinatorial approach of BRAFi plus anti-Nodal mAb, compared with monotherapy and control. These data provide a promising new strategic approach using front-line therapy together with targeting a CSC-associated molecule—Nodal.

Conclusion

The heterogeneity and plasticity of aggressive melanoma present formidable challenges in the design of current therapies. However, recognizing that cancer cells can reactivate normally dormant embryonic pathways to exacerbate tumorigenicity and metastasis may present a unique therapeutic opportunity. The multipotent

phenotype of aggressive melanoma cells—with vascular, embryonic, and CSC capabilities—offers new insights into their functional adaptation and resistance to current therapies. Considering that aggressive tumors utilize multiple mechanisms to survive and metastasize, it seems prudent to use evidence-based reports to develop combinatorial strategies to effectively target heterogeneous melanoma subpopulations—to eliminate relapse and disease progression.

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References

1. Bittner M, Meltzer P, Chen Y, Jiang Y, Seftor E, Hendrix MJC, et al. Molecular classification of cutaneous malignant melanoma by gene expression profiling. Nature. 2000 Aug 3;406(6795):536–40. http://dx.doi.org/10.1038/35020115
2. Song X, Zhao Z, Barber B, Farr AM, Ivanov B, Novish M. Overall survival in patients with metastatic melanoma. Curr Med Res Opin. 2015 May;31(5):987–91. http://dx.doi.org/10.1185/03007995.2015.1021904
3. Gogas HJ, Kirkwood JM, Sondak VK. Chemotherapy for metastatic melanoma: Time for a change. Cancer 2007 Feb 1;109:455–64. http://dx.doi.org/10.1002/cncr.22427
4. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010 Aug 19;363:711–23. http://dx.doi.org/10.1056/NEJMoa1003466
5. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med. 2011 Jun 30;364:2507–16. http://dx.doi.org/10.1056/NEJMoa1103782
6. Ascierto PA, Minor D, Ribas A, Lebbe C, O'Hagan A, Arya N, et al. Phase II trial (BREAK-2) of the BRAF inhibitor dabrafenib (GSK2118436) in patients with metastatic melanoma. J Clin Oncol 2013 Sept 10;31:3205–11. http://dx.doi.org/10.1200/JCO.2013.49.8691
7. Flaherty KT, Robert C, Hersey P, Nathan P, Garbe C, Milhem M, et al. Improved survival with MEK inhibition in BRAF-mutated melanoma. N Engl J Med 2012 Jul 12;367:107–14. http://dx.doi.org/10.1056/NEJMoa1203421
8. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of PD-1 antibody in cancer. N Engl J Med. 2012 Jun 28;366:2443–54. http://dx.doi.org/10.1056/NEJMoa1200690
9. Folkman J. Seminars in Medicine of the Beth Israel Hospital, Boston. Clinical applications of research on angiogenesis. N Engl J Med. 1995 Dec 28;333:1757–63. http://dx.doi.org/10.1056/NEJM199512283332608
10. Seftor EA, Meltzer PS, Schatteman GC, Gruman LM, Hess AR, Kirschmann DA, et al. Expression of multiple molecular phenotypes by aggressive melanoma tumor cells: Role in vasculogenic mimicry. Crit Rev Oncol Hematol. 2002 Oct;44:12–27. http://dx.doi.org/10.1016/S1040-8428(01)00199-8

11. Hendrix MJC, Seftor EA, Meltzer PS, Gardner LM, Hess AR, Kirschmann DA, et al. Expression and functional significance of VE-cadherin in aggressive human melanoma cells: Role in vasculogenic mimicry. Proc Natl Acad Sci U S A. 2001 Jul 3;98:8018–23. http://dx.doi.org/10.1073/pnas.131209798
12. Hendrix MJC, Seftor EA, Hess AR, Seftor REB. Vasculogenic mimicry and tumour-cell plasticity: Lessons from melanoma. Nat Rev Cancer. 2003 Jun;3(6):411–21. http://dx.doi.org/10.1038/nrc1092
13. Seftor REB, Hess AR, Seftor EA, Kirschmann DA, Hardy KM, Margaryan NV, et al. Tumor cell vasculogenic mimicry: From controversy to therapeutic promise. Am J Pathol. 2012 Oct;181(4):1115–25. http://dx.doi.org/10.1016/j.ajpath.2012.07.013
14. Yang JP, Liao YD, Mai DM, Xie P, Qiang YY, Sheng LS, et al. Tumor vasculogenic mimicry predicts poor prognosis in cancer patients: A meta-analysis. Angiogenesis. 2015 Oct 25;19:191–200. http://dx.doi.org/10.1007/s10456-016-9500-2
15. Maniotis AJ, Folberg R, Hess AR, Seftor EA, Gardner LM, Pe'er J, et al. Vascular channel formation by human melanoma cells in vivo and in vitro: Vasculogenic mimicry. Am J Path. 1999 Sept;155(3):739–52. http://dx.doi.org/10.1016/S0002-9440(10)65173-5
16. McDonald DM, Munn L, Jain RK. Vasculogenic mimicry: How convincing, how novel, and how significant? Am J Pathol. 2000 Feb;156(2):383–8. http://dx.doi.org/10.1016/S0002-9440(10)64740-2
17. Van de Schaft D, Seftor REB, Seftor EA, Hess AR, Gruman LM, Kirschmann DA, et al. Effects of angiogenesis inhibitors on vascular network formation by human endothelial and melanoma cells. J Natl Cancer Inst. 2004 Oct 6;96(19):1473–77. http://dx.doi.org/10.1093/jnci/djh267
18. Fidler IJ, Ellis LM. Neoplastic angiogenesis—Not all blood vessels are created equal. N Engl J Med. 2004 Jul 15;351:215–16. http://dx.doi.org/10.1056/NEJMp048080
19. Demou ZN, Hendrix MJC. Microgenomics profile the endogeneous angiogenic phenotype in subpopulations of aggressive melanoma. J Cell Biochem. 2008 Oct 1;105:562–73. http://dx.doi.org/10.1002/jcb.21855
20. Zhou Y, Fisher SJ, Janatpour M, Genbacev O, Dejana E, Wheelock M, et al. Human cytotrophoblasts adopt a vascular phenotype as they differentiate. J Clin Invest. 1997 May;99(9):2139–51. http://dx.doi.org/10.1172/JCI119387
21. Topczewska JM, Postovit LM, Margaryan NV, Sam A, Hess AR, Wheaton WW, et al. Embryonic and tumorigenic pathways converge via Nodal signaling: Role in melanoma aggressiveness. Nature Med. 2006 Aug;12(8):925–32. http://dx.doi.org/10.1038/nm1448
22. Postovit LM, Margaryan NV, Seftor EA, Kirschmann DA, Lipavsky A, Wheaton WW, et al. Human embryonic stem cell microenvironment suppresses the tumorigenic phenotype of aggressive cells. Proc Natl Acad Sci U S A. 2008 Mar 18;105(11):4329–34. http://dx.doi.org/10.1073/pnas.0800467105
23. Schier AF. Nodal signaling in vertebrate development. Annu Rev Cell Dev Biol. 2003;19:589–621. http://dx.doi.org/10.1146/annurev.cellbio.19.041603.094522
24. Costa FF, Seftor EA, Bischof JM, Kirschmann DA, Strizzi L, Arndt K, et al. Epigenetically reprogramming metastatic tumor cells with an embryonic microenvironment. Epigenomics. 2009 Dec;1(2):387–98. http://dx.doi.org/10.2217/epi.09.25
25. Strizzi L, Sandomenico A, Margaryan NV, Foca A, Sanguigno L, Bodenstine TM, et al. Effects of a novel Nodal-targeting monoclonal antibody in melanoma. Oncotarget. 2015 Oct 9;6(33):34071–86.
26. Strizzi L, Hardy KM, Margaryan NV, Hillman DW, Seftor EA, Chen B, et al. Potential for the embryonic morphogen Nodal as a prognostic and predictive biomarker in breast cancer. Breast Cancer Res. 2012 May 11;14(3):R75. http://dx.doi.org/10.1186/bcr3185
27. Foca A, Sanguigno L, Foca G, Strizzi L, Iannitti R, Palumbo R, et al. New anti-Nodal monoclonal antibodies targeting the Nodal pre-helix loop involved in Cripto-1 binding. Int J Mol Sci. 2015 Sep 7;16(9):21342–62. http://dx.doi.org/10.3390/ijms160921342
28. Strizzi L, Postovit LM, Margaryan NV, Lipavsky A, Gadiot J, Blank C, et al. Nodal as a biomarker for melanoma progression and a new therapeutic target for clinical intervention. Expert Rev Dermatol. 2009;4(1):67–78. http://dx.doi.org/10.1586/17469872.4.1.67
29. Lai CY, Schwartz BE, Hsu MY. CD133+ melanoma subpopulations contribute to perivascular niche morphogenesis and tumorigenicity through vasculogenic mimicry. Cancer Res. 2012 Oct 1;72(19):5111–18. http://dx.doi.org/10.1158/0008-5472.CAN-12-0624

30. Seftor EA, Seftor REB, Weldon DS, Kirsammer GT, Margaryan NV, Gilgur A, et al. Semin Oncol. 2014 Apr;41(2):259–66. http://dx.doi.org/10.1053/j.seminoncol.2014.02.001
31. Hardy KM, Strizzi L, Margaryan NV, Gupta K, Murphy GF, Scolyer RA, et al. Targeting Nodal in conjunction with Dacarbazine induces synergistic anticancer effects in metastatic melanoma. Mol Cancer Res. 2015 Apr;13(4):670–80. http://dx.doi.org/10.1158/1541-7786.MCR-14-0077
32. Hendrix MJC, Kandela I, Mazar AP, Seftor EA, Seftor REB, Margaryan NV, et al. Targeting melanoma with front-line therapy does not abrogate Nodal-expressing tumor cells. Lab Invest. 2017 Feb;97(2):176–86. http://dx.doi.org/10.1038/labinvest.2016.107

5 Ulcerated Melanoma: Aspects and Prognostic Impact

MARIE LOUISE BØNNELYKKE-BEHRNDTZ[1] • TORBEN STEINICHE[2]

[1]Department of Plastic and Reconstructive Surgery, Aarhus University Hospital, Aarhus, Denmark; [2]Department of Pathology, Aarhus University Hospital, Aarhus, Denmark

Author for correspondence: Marie Louise Bønnelykke-Behrndtz, Department of Plastic and Reconstructive Surgery, Aarhus University Hospital, Aarhus, Denmark. E-mail: mariboen@rm.dk; louiseboennelykke@gmail.com

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Abstract: Ulceration is an important prognostic factor for patients with melanoma and also a predictive marker for the response of adjuvant immune-stimulating therapy. A consensual definition and accurate assessment of ulceration is therefore crucial for proper staging and clinical management, but can be difficult even between experienced pathologists. The definition of ulceration is stated differently in the available literature but is generally understood as loss of epidermal matrix. Thinning of the epidermis, also termed consumption of the epidermis (COE), is associated with ulcerated lesions and correlates with enhanced tumor cell proliferation in nonulcerated melanoma. These results suggest that COE may be a proliferative precursor of ulceration, characterized by erosive growth into the epidermal layer (infiltrative type) or expansive growth that may stretch and eventually disrupt the epidermis (attenuative type), which is reflected in the histopathology. We have no means to determine the dynamic changes of human ulcerated melanoma or to determine whether these wounds have re-epithelialization (RE) potential. However, the presence of reactive hyperplasia (REH) and changes indicating RE associates with increased density of neutrophils and may herald resolved or late-stage ulcerations. Combining the extent of ulceration (> or <70% of the total tumor length) and the presence of

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epidermal involvement (COE, REH, and/or RE) stratifies prognosis more accurately and supports the relevance of including these factors in the definition of ulceration and to define ulceration of a primary melanoma as loss of epidermis with evidence of a host response (infiltration of neutrophils or fibrin deposition) and thinning, effacement, or REH of the surrounding epidermis.

Key words: Consumption of epidermis; Neutrophils; Prognosis; Proliferation; Ulceration

Introduction

Patients with ulcerated melanoma form a subgroup with shorter disease-free and overall survival than patients with nonulcerated melanoma (1, 2). The presence of ulceration upstages patients with localized melanoma by both subcategories and stages and is included as an independent prognostic factor defining the T-stage in the American Joint Committee of Cancer's melanoma staging criteria (1, 2). Patients with ulceration form a subgroup whose survival is significantly improved when they are treated with adjuvant immunotherapy (3, 4) compared with observation alone. Accurate assessment of ulceration is therefore crucial for proper staging and clinical management but can be difficult even for experienced pathologists. There is currently no evidence-based definition of ulceration and no consensus in the published literature. The American Joint Committee on Cancer (AJCC) has defined ulceration as the absence of an intact epidermis overlying a major portion of the primary melanoma based on microscopic examination of the histologic sections (5). Other studies have defined ulceration as full-thickness loss of epidermis associated with a host reaction (infiltration of neutrophils and/or fibrin deposition) (6). Interestingly, a former study showed that the interobserver reproducibility increased by defining ulceration as full-thickness epidermal defect (including absence of stratum corneum and basement membrane), with evidence of a host response (i.e., fibrin deposition, neutrophils) and thinning, effacement, or reactive hyperplasia (REH) of the surrounding epidermis (7).

In this chapter, ulceration is defined as full-thickness loss of epidermis overlying melanoma tissue, in which epidermal loss was associated with a host reaction (infiltration of neutrophils and/or fibrin deposition) (8). Characteristics of the surrounding epidermis were coded and analyzed separately, aimed at a better understanding of the biology and impact of these changes, allowing better stratification of ulcerated melanoma.

Consumption of the Epidermis:A Possible Precursor of Ulceration That Associates with Increased Tumor Cell Proliferation

Cleft formation (CF, gap formation in the dermal/epidermal junction) and consumption of epidermis (COE, general thinning of the epidermis) are interesting phenomena as they may indicate early structural changes and be possible

precursors of ulceration. CF correlates with an increased Breslow thickness and to the presence of ulceration (8). However, either the presence, type, or the extent of CF had prognostic impact (8). The visualized CF could be due to several factors: artifacts, increased proliferation and thereby erosion of hemi-desmosomes, or cell–cell adhesion loss. Nineteen percent of the tumors displaying CF are sealed with CD34-positive endothelial cells in the dermal/epidermal junction (9). This indicates that the presence of CF could also be due to blocked and dilated vessels of the superficial plexus. In 72% of the tumors, CF associates with infiltrative epidermal growth of melanoma cells and focal thinning of the overlying epidermis, which may be a possible precursor of focal ulceration (9).

COE has been defined as thinning of the epidermis—attenuation of basal and suprabasal layers and loss of the normal rete-ridge configuration in areas overlying melanoma tissue (10)—and its presence is reported in between 37 and 86% of all melanomas (8, 10–12) (Figure 1). COE can be detected in thin melanomas, but its likelihood rises with increasing Breslow thickness, and only 18% of thin melanomas (<1 mm) and 46% of the thicker melanomas (2–4 mm) had COE (8). There was a strong correlation between COE and ulceration, with 25% of nonulcerated melanomas and 52% of the ulcerated tumors showing presence of COE (8). These figures are in line with those reported in other studies (11, 13). Consumption was first defined and introduced as an important factor for differentiating melanomas from Spitz nevi (10), whereas Walters et al. showed a correlation between COE and ulceration (11). In this latter study, COE was frequently found at the edges of ulcerated areas and it was thought of as an early step in the progression toward ulceration (8).

The biology behind COE is not clear, but theoretically thinning of the epidermis may be tumor cells proliferating into the epidermis (infiltrative type) or expansive growth that stretches the epidermis thin (attenuative type) (8, 14) (Figure 2). Supporting these hypothesis, tumors with COE displayed 37% increased tumor cell proliferation compared with tumors of normal epidermal configuration (9), when only the nonulcerated melanomas are analyzed. COE was associated with increased Breslow thickness, nodular melanoma, and age over 50 years, but after adjusting for these factors the proliferative index in tumors with COE was still significantly increased (9). It is therefore suggested that COE is a proliferative precursor of ulceration, in which increased proliferation may either erode or stretch the epidermis thin and finally ulcerate it (14). There was no increased inflammatory response (cd163+ macrophages or cd66b+ neutrophils) associated with COE, supporting a noninflammatory drive of proliferation (9). In contrast with this possible erosion of epidermis, total loss of epidermis and an ulcer are associated with a robust inflammation response (15), with a vital reaction with neutrophils or fibrin being suggested as important in the definition of ulceration.

Melanomas with Re-Epithelialization and Reactive Epidermal Hyperplasia May Herald Late-Stage or Resolved Ulcerations

The presence of a thin epidermis under or at the edges of a scab can be seen as a possible instance of re-epithelialization (RE), and enlargement of epidermis or elongated rete-ridges as reactive epidermal hyperplasia (8) (Figure 1).

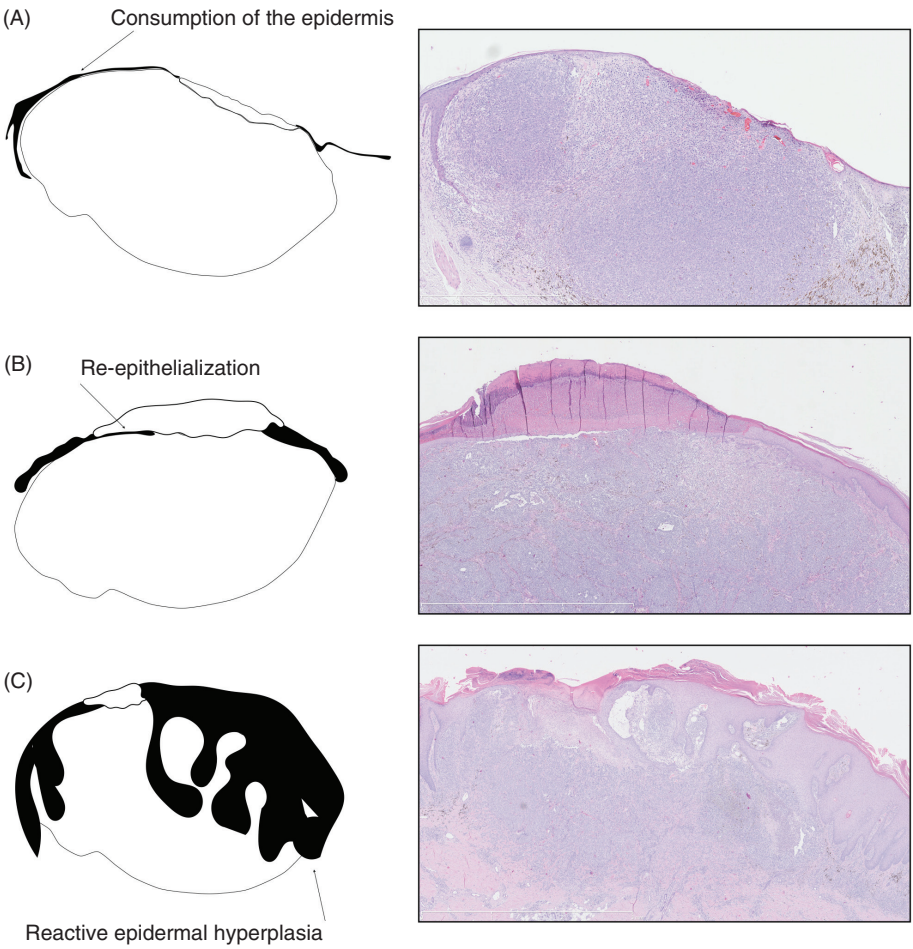

Figure 1 An illustration of the different types of epidermal involvement. (A) Consumption of the epidermis, defined as general epidermal thinning (involving >2/3 of the epidermis) and loss of rete-ridge configuration in areas with direct contact to underlying melanoma tissue. (B) Possible re-epithelialization, a thin, few-layered epidermis under or at the edges of a scab. (C) Reactive epidermal hyperplasia, enlargement of epidermis, with increased epidermal layers and elongated rete-ridges. (Adapted from Am J Clin Pathol 2014;142(6):845–856.)

These phenomena are described phases during wound healing, seen subsequent to ulceration and wounding (16). A vital reaction with neutrophils and/or fibrin and the presence of epidermal changes may therefore be important characteristics distinguishing tumor-induced ulceration from traumatic disruption of epidermis, related to the surgical or preparation procedure. Scratching as a traumatic ulceration may be impossible to distinguish from tumor-induced ulceration though, as these lesions would present a vital reaction of the underlying melanoma tissue, with unknown biological and prognostic impact. There is no means to determine

the dynamic changes of human ulcerated melanoma or whether these wounds possess the ability of RE. However, the presence of REH and changes indicating RE has been found associated with ulceration and observed in 13% of melanoma (8). In addition, the density of cd66b+ neutrophils was increased by 18% in tumors presenting RE and reactive epidermal hyperplasia compared with tumors with no evidence of the described characteristics (9). It therefore seems reasonable to suggest that these melanomas may possess prolonged, late-stage, or resolved ulcerations.

Ulceration Is a Heterogeneous Phenomenon of Which Both the Type and Extent Matters

Studies have found that the extent of ulceration seemed to stratify prognosis more accurately than the mere presence or absence of ulceration (6, 17–19). In a recent study of localized melanoma, Hout et al. subdivided ulcerated melanoma into excessive (>5 mm or >70%) or minimal/moderate ulcerations (<5 mm or <70%). This provided additional prognostic information, and showed that excessive ulceration had a significantly negative effect on melanoma-specific survival (6). In line with this, a detailed analysis of ulcerated melanomas in 179 patients compared with 207 patients with nonulcerated melanomas found that excessive ulceration, measured as percentage of the total tumor length (>70%), was an independent predictor of poor survival compared with minimal/moderate ulceration (<70%) (8).

While the prognostic impact of the extent of ulceration is supported by the literature, the prognostic impact of the type of ulceration is less clear. Two different types of ulceration have been described; an infiltrative type in which infiltrative growth erodes the epidermal layer or an attenuative type, defined as expansive growth, that stretches and eventually disrupts the epidermal layer (14) (Figure 2). One study reported that an attenuative type of ulceration is an independent predictor of poor melanoma-specific survival as compared with both an infiltrative type and nonulcerated lesions (8). The histological type (superficial spreading vs. nodular) was found to have no independent prognostic value; however, it correlated significantly with the type of ulceration (8). After adjustment of the histological type, an attenuative type of ulceration retained its independent significance (8). Combining the described pattern of ulceration with the presence of COE, an attenuative type of consumption demonstrated independent prognostic value, in line with the prognostic impact of the attenuative type of ulceration (8). Fair to good interobserver reproducibility of the type of ulceration is reported; however, in a clinical setting, this marker might be difficult to implicate (8). Distinction between the different patterns of epidermal infiltration is interesting, though, as it may reflect important differences in the biological nature or tumor microenvironments. Infiltrative ulceration has been characterized by increased and erosive growth into the epidermal layer, which may disrupt cellular adhesion (14, 20). This is in contrast to melanomas with an attenuative type of ulceration which show minimal epidermal erosion; however, expansive growth of melanomas may stretch and eventually disrupt the epidermis (14, 20). Cramer et al. suggest that intraepidermal growth and erosion may be a marker of more mature melanoma cells as opposed to more immature and dermal oriented melanoma cells (21).

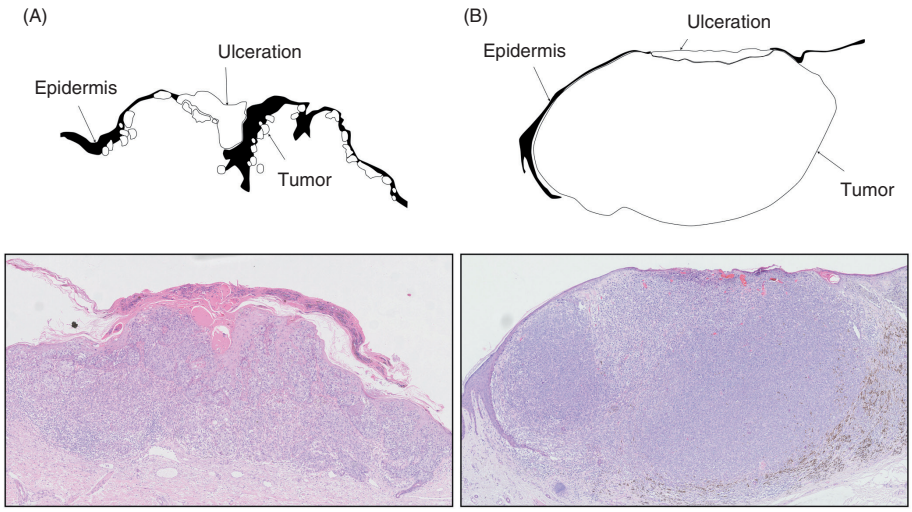

Figure 2 An illustration of the type of epidermal involvement and ulceration in melanoma. (A) Infiltrative ulceration with melanoma cells that infiltrate and erode the epidermal layer. (B) Attenuative ulceration where nodular growth stretches and eventually disrupts the epidermal layer, with minimal epidermal infiltration. (Adapted from Am J Clin Pathol 2014;142(6):845–856.)

The Extent of Ulceration and Changes of the Surrounding Epidermis Have a Prognostic Impact

AJCC has defined ulceration as the absence of an intact epidermis overlying a major portion of the primary melanoma based on microscopic examination of the histologic sections (1, 5). However, by including full-thickness epidermal loss, with evidence of a host response and thinning, effacement, or REH of the surrounding epidermis into the definition of ulceration the interobserver reproducibility increased (6). The described association between the biological markers (proliferation and inflammation) and the prognostic impact of the histopathological changes further supports the relevance of including epidermal changes into the definition.

Combining the presence of epidermal involvement (COE, RE, or reactive epidermal hyperplasia) and the extent of ulceration, patients with a normal epidermis and patients with minimal/moderate ulceration without epidermal involvement have equivalent 5-year survival rates, while patients with minimal/moderate ulceration with epidermal involvement and patients with excessive ulcerations, both of which are independent prognostic factors, have significantly poorer survival in multivariate analysis (8) (Figure 3). The extent of ulceration and involvement of the surrounding epidermis (COE, RE, and reactive epidermal hyperplasia) is therefore suggested as a useful marker allowing better stratification of ulcerated melanoma.

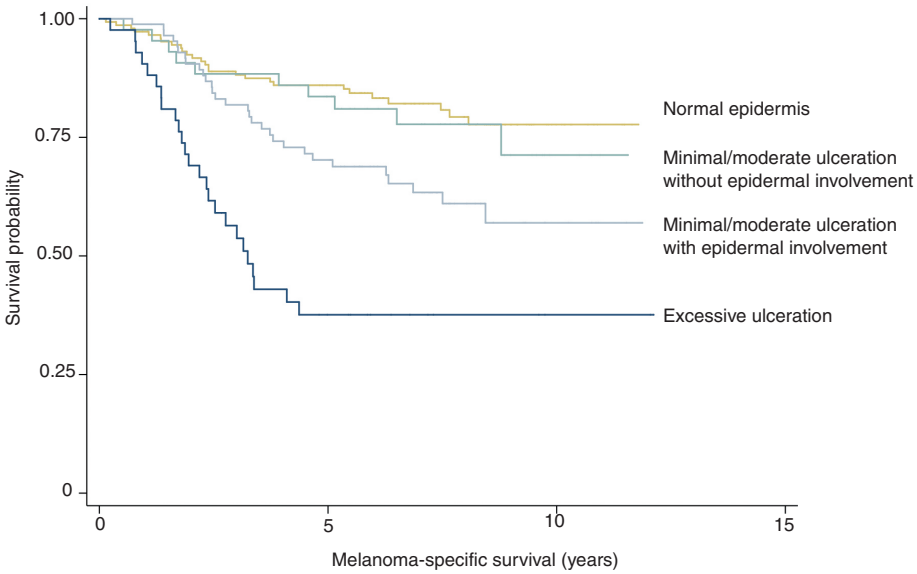

Figure 3 Kaplan–Meier survival curves, illustrating the prognostic impact of the extent of ulceration and the involvement of the surrounding epidermis. Combining the extent of ulceration (as percentage of the ulceration length over the total tumor length, > or <70%) and epidermal involvement (presence of either consumption of epidermis, re-epithelialization, or reactive epidermal hyperplasia) provided additional prognostic information. (Adapted from Am J Clin Pathol 2014;142(6):845–856.)

In conclusion, a consensual definition of ulceration is pivotal for proper staging, and clinical management and ulceration is defined as full-thickness loss of epidermal matrix, with evidence of an underlying host reaction (infiltration of neutrophils) and thinning (COE), effacement (RE), or REH of the surrounding epidermis.

Conclusion

Ulceration is an important prognostic factor for patients with melanoma and interestingly also a predictive marker for the response of adjuvant immune-stimulating therapy. A consensual definition and accurate assessment of ulceration is therefore crucial for proper staging and clinical management. COE, defined as thinning of epidermis, involving >2/3 of the epidermis correlated with increased levels of tumor cell proliferation (Ki67/MelanA) compared with tumors demonstrating normal epidermal configuration and is suggested as a proliferative precursor of ulceration. We have no means to determine the dynamic changes of human ulcerated melanoma or to determine whether these wounds have a RE potential. However, the presence of reactive hyperplasia, and changes indicating re-epithelialization, associated significantly with increased density of cd66b+ neutrophils when compared with tumours that have no evidence of these changes; this may indicate prolonged, late-stage or resolved ulcerations. An attenuative type of epidermal

involvement thought of as expansive growth that stretches the epidermis thin and eventually causes disruption was independently linked with poor melanoma-specific survival, in contrast to an infiltrative type that may erode the epidermis thin and leave it ulcerated. The type of ulceration may have an interesting biological explanation but is more difficult to implement in a clinical setting. The extent of ulceration (including >70% of the tumor length) and involvement of the surrounding epidermis (COE, reactive epidermal hyperplasia, and RE) provided more accurate prognostic information than the mere absence or presence and is suggested to be useful markers allowing better stratification of ulcerated lesions.

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References

1. Balch CM, Gershenwald JE, Soong SJ, Thompson JF, Atkins MB, Byrd DR, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol. 2009 Dec 20;27(36):6199–206. http://dx.doi.org/10.1200/JCO.2009.23.4799
2. Balch CM, Gershenwald JE, Soong SJ, Thompson JF. Update on the melanoma staging system: The importance of sentinel node staging and primary tumor mitotic rate. J Surg Oncol. 2011 Sep;104(4):379–85. http://dx.doi.org/10.1002/jso.21876
3. Eggermont AM, Suciu S, Testori A, Kruit WH, Marsden J, Punt CJ, et al. Ulceration and stage are predictive of interferon efficacy in melanoma: Results of the phase III adjuvant trials EORTC 18952 and EORTC 18991. Eur J Cancer. 2012 Jan;48(2):218–25. http://dx.doi.org/10.1016/j.ejca.2011.09.028
4. Eggermont AM, Chiarion-Sileni V, Grob JJ, Dummer R, Wolchok JD, Schmidt H, et al. Adjuvant ipilimumab versus placebo after complete resection of high-risk stage III melanoma (EORTC 18071): A randomised, double-blind, phase 3 trial. Lancet Oncol. 2015 May;16(5):522–30. http://dx.doi.org/10.1016/S1470-2045(15)70122-1
5. Balch CM, Buzaid AC, Soong SJ, Atkins MB, Cascinelli N, Coit DG, et al. Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. J Clin Oncol. 2001 Aug 15;19(16):3635–48. http://dx.doi.org/10.1200/JCO.2001.19.16.3635
6. In 't Hout FE, Haydu LE, Murali R, Bonenkamp JJ, Thompson JF, Scolyer RA. Prognostic importance of the extent of ulceration in patients with clinically localized cutaneous melanoma. Ann Surg. 2012 Jun;255(6):1165–70. http://dx.doi.org/10.1097/SLA.0b013e31824c4b0b
7. Spatz A, Cook MG, Elder DE, Piepkorn M, Ruiter DJ, Barnhill RL. Interobserver reproducibility of ulceration assessment in primary cutaneous melanomas. Eur J Cancer. 2003 Sep;39(13):1861–5. http://dx.doi.org/10.1016/S0959-8049(03)00325-3
8. Bonnelykke-Behrndtz ML, Schmidt H, Christensen IJ, Damsgaard TE, Moller HJ, Bastholt L, et al. Prognostic stratification of ulcerated melanoma: Not only the extent matters. Am J Clin Pathol. 2014 Dec;142(6):845–56. http://dx.doi.org/10.1309/AJCPW56PHGLFTKZC
9. Bonnelykke-Behrndtz LM, Schmidt H, Damsgaard TE, Christensen IJ, Bastholt L, Moller HJ, et al. Consumption of the epidermis: A suggested precursor of ulceration associated with increased proliferation of melanoma cells. Am J Dermatopathol. 2015 Nov;37(11):841–5. http://dx.doi.org/10.1097/DAD.0000000000000382

10. Hantschke M, Bastian BC, LeBoit PE. Consumption of the epidermis: A diagnostic criterion for the differential diagnosis of melanoma and Spitz nevus. Am J Surg Pathol. 2004 Dec;28(12):1621–5. http://dx.doi.org/10.1097/00000478-200412000-00011
11. Walters RF, Groben PA, Busam K, Millikan RC, Rabinovitz H, Cognetta A, et al. Consumption of the epidermis: A criterion in the differential diagnosis of melanoma and dysplastic nevi that is associated with increasing Breslow depth and ulceration. Am J Dermatopathol. 2007 Dec;29(6):527–33. http://dx.doi.org/10.1097/DAD.0b013e318156e0a7
12. Seckin S, Ozgun E. The importance of consumption of the epidermis in malignant melanoma and correlation with clinicopathological prognostic parameters. Turk Patoloji Derg. 2011 Jan;27(1):51–6. http://dx.doi.org/10.5146/tjpath.2010.01047
13. Ohata C, Nakai C, Kasugai T, Katayama I. Consumption of the epidermis in acral lentiginous melanoma. J Cutan Pathol. 2012 Jun;39(6):577–81. http://dx.doi.org/10.1111/j.1600-0560.2012.01914.x
14. Scolyer RA, Shaw HM, Thompson JF, Li LX, Colman MH, Lo SK, et al. Interobserver reproducibility of histopathologic prognostic variables in primary cutaneous melanomas. Am J Surg Pathol. 2003 Dec;27(12):1571–6. http://dx.doi.org/10.1097/00000478-200312000-00011
15. Antonio N, Bonnelykke-Behrndtz ML, Ward LC, Collin J, Christensen IJ, Steiniche T, et al. The wound inflammatory response exacerbates growth of pre-neoplastic cells and progression to cancer. EMBO J. 2015 Sep 2;34(17):2219–36. http://dx.doi.org/10.15252/embj.201490147
16. Martin P. Wound healing—Aiming for perfect skin regeneration. Science. 1997 Apr 4;276(5309):75–81. http://dx.doi.org/10.1126/science.276.5309.75
17. Balch CM, Wilkerson JA, Murad TM, Soong SJ, Ingalls AL, Maddox WA. The prognostic significance of ulceration of cutaneous melanoma. Cancer. 1980 Jun 15;45(12):3012–17. http://dx.doi.org/10.1002/1097-0142(19800615)45:12%3C3012::AID-CNCR2820451223%3E3.0.CO;2-O
18. Day CL Jr, Lew RA, Harrist TJ. Malignant melanoma prognostic factors 4: Ulceration width. J Dermatol Surg Oncol. 1984 Jan;10(1):23–4. http://dx.doi.org/10.1111/j.1524-4725.1984.tb01167.x
19. Day CL Jr, Harrist TJ, Gorstein F, Sober AJ, Lew RA, Friedman RJ, et al. Malignant melanoma. Prognostic significance of "microscopic satellites" in the reticular dermis and subcutaneous fat. Ann Surg. 1981 Jul;194(1):108–12. http://dx.doi.org/10.1097/00000658-198107000-00019
20. Azzola MF, Shaw HM, Thompson JF, Soong SJ, Scolyer RA, Watson GF, et al. Tumor mitotic rate is a more powerful prognostic indicator than ulceration in patients with primary cutaneous melanoma: An analysis of 3661 patients from a single center. Cancer. 2003 Mar 15;97(6):1488–98. http://dx.doi.org/10.1002/cncr.11196
21. Cramer SF, Consumption of the epidermis – What is its place in the spectrum of aberrant melanocyte-keratinocyte interactions? Am J Dermatopathol. 2008 Apr;30(2):200–3. http://dx.doi.org/10.1097/DAD.0b013e318164ede6

Section II

Therapy and Management

6 Clinical Presentation and Staging of Melanoma

WILLIAM H. WARD[1] • FERNANDO LAMBRETON[1] • NEHA GOEL[1] • JIAN Q. YU[2] • JEFFREY M. FARMA[1]

[1]Department of Surgical Oncology, Fox Chase Cancer Center, Philadelphia, PA, USA; [2]Department of Diagnostic Imaging, Fox Chase Cancer Center, Philadelphia, PA, USA

Author for correspondence: William H. Ward, Department of Surgical Oncology, Fox Chase Cancer Center, Philadelphia, PA, USA; Email: william.ward@fccc.edu

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Abstract: Cutaneous melanoma is responsible for the vast majority of skin cancer-related deaths in the United States. Known risk factors include genetic defects, environmental exposures, and a combination of both. Among environmental risks, exposure to ultraviolet rays is the most important and the most modifiable risk factor. Several genetic syndromes involve increased risk of melanoma, including xeroderma pigmentosum, familial atypical multiple moles and melanoma syndrome, BRCA2 mutation, and congenital melanocytic nevi. Although the necessity of implementation remains controversial, the most effective melanoma screening technique is the whole-body skin examination. Typically, melanoma lesions are incidentally discovered during routine skin examination using the "ABCDE" mnemonic. Once suspected, questions pertaining to the sites of potential metastasis should be asked and excisional or partial biopsy should be considered. The primary histologic subtypes of melanoma include superficial spreading, lentigo maligna, nodular, acral lentiginous, desmoplastic, and amelanotic. Melanoma staging is completed via clinical and histologic assessment using the American Joint Committee on Cancer TNM system. Delayed or deficient elements of initial melanoma evaluation can limit patient outcomes and increase

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disease-related mortality. Clinicians involved in the diagnosis or treatment of cutaneous melanoma must be familiar with the available screening options, key steps of diagnosis, and the staging ramifications of disease discovery.

Key words: ABCDE system; Clinical presentation; Diagnostic strategies; Melanoma; Staging

Introduction

Despite the important progress seen in the treatment of oncologic diseases over the past few decades, the incidence and mortality associated with malignant melanoma continues to increase (1). Among the most common malignancies in the United States, the incidence of melanoma currently ranks fifth overall when compared to other common cancers (2). As a result of its aggressive behavior and diagnostic challenges, it is responsible for the vast majority of skin cancer-related deaths. This chapter will focus on appropriate screening considerations for melanoma, clinical approaches to diagnosis and confirmation, and updated staging guidelines to facilitate subsequent therapy (1).

Screening Considerations

ETIOLOGY OF DISEASE DEVELOPMENT

Cutaneous melanoma evolves from aberrant melanocytes located within the basal layer of the epidermis. These melanocytes are responsible for the production of melanin, a substance which absorbs potentially harmful ultraviolet (UV) radiation. Left unchecked, UV radiation affects integumentary cells by causing direct damage to individual DNA strands. Although UV-induced DNA damage is normally repaired by specific DNA repair mechanisms, genetic or environmentally derived errors within this repair complex can lead to the formation of an invasive melanoma (3, 4).

GENERAL RISK FACTORS

Like most other neoplastic conditions, known risk factors of melanoma include genetic defects, environmental exposure, and a combination of both (5). Although multiple genetic syndromes incur a significantly increased risk for the development of cutaneous malignancy (discussed later), inherited phenotypic traits associated with melanoma include fair skin, light hair, red hair, freckles, and light eye color. Unsurprisingly, a positive family history is a strong risk factor for the evolution of this disease. As the number of first-degree relatives with melanoma increases, so does the risk of developing the disease (6). Patients with one first-degree relative with melanoma are 1.7 times more likely to be diagnosed with melanoma, whereas two first-degree relatives incur a nine-fold increase in risk. In addition, as patients with a positive family history grow older, the cumulative risk of melanoma also increases (7).

Regarding environmental risks, UV exposure is the most important and the most potentially modifiable risk factor contributing to the development of melanoma. Compared with those with chronic and continuous exposure, patients with intermittent, more intense exposure to the sun are at much higher risk (4). A history of sunburns, specifically blistering sunburns in childhood and adulthood, can be associated with approximately twice the baseline risk of melanoma development (5). Significant UV radiation exposure before the age of 35 significantly increases the risk of melanoma (7). Although UV-A sunlight has certainly been implicated as a cause of melanoma (e.g., tanning salon-related UV radiation), most skin damage is actually caused by UV-B rays (4).

Chronic immunosuppression represents another exposure-related risk factor for melanoma development. Such immunosuppression may be the result of an existing neoplastic condition. For example, approximately 5% of patients with a personal history of melanoma will be diagnosed with a second melanoma (6). In addition, patients with a personal history of nonmelanoma skin cancer have more than a fourfold relative risk of developing melanoma. Other causes of chronic immunosuppression may result from pharmaceutical agents used in the treatment of AIDS, chronic lymphocytic leukemia, non-Hodgkin's lymphoma, or patients with organ transplantation (7).

POPULATIONS AT INCREASED RISK

As discussed previously, several genetic syndromes involve a significantly increased risk of melanoma development. These conditions include xeroderma pigmentosum (XP), familial atypical multiple moles and melanoma (FAMMM) syndrome, BRCA2 mutation, and congenital melanocytic nevi (7, 8). XP, an autosomal recessive condition in which UV-related DNA repair mechanisms are deficient, carries an approximately 1000-fold increase in the risk of melanoma. Sun avoidance and regular self-skin examinations are mandatory, as is frequent surveillance by a dermatologist with extensive XP experience (6, 7).

FAMMM syndrome, also known as the B-K mole syndrome, is caused by germline mutations in CDKN2A (6). An autosomal dominant condition, FAMMM syndrome has incomplete penetrance. Diagnosis is determined through family history and is confirmed when at least two first-degree relatives have both melanoma and multiple dysplastic nevi. Interestingly, overall survival is similar to that of sporadic melanoma (7). Families with a suspected diagnosis of FAMMM should undergo frequent skin examinations and should complete a genetics consultation to evaluate for CDKN2A mutation. As CDKN2A mutations are also associated with pancreatic cancer, extensive documentation of the family history is mandatory in these patients, and screening for other associated malignancies should occur (6).

More widely associated with inherited breast and ovarian carcinomas, a BRCA2 mutation nearly triples the risk of cutaneous melanoma development. A tumor suppressor gene, mutations in BRCA2 also degrade cellular DNA repair mechanisms. As BRCA2 mutations can also lead to prostate and pancreatic cancers, potential patients should similarly undergo genetics or risk assessment evaluations following the documentation of a thorough family history (8).

The presence of congenital melanocytic nevi also increases the risk of melanoma development, with larger lesions having the highest risk. These lesions can

be either followed closely or removed prophylactically. Since melanomas that occur within congenital melanocytic nevi usually develop before the age of 10, prophylactic removal of these lesions should be considered early in life (7).

SCREENING RECOMMENDATIONS

The most effective melanoma screening technique is the whole-body skin examination (WBE). WBE involves a review of the entire cutaneous surface of a disrobed patient by the treating provider. Despite the proven efficacy of this approach, completion of this screening technique is less common than preventative screening modalities used in the early detection of other malignancies. The implementation of the WBE as an annual melanoma screening tool in the United States has been controversial (1, 9). Although many within the dermatology and oncology communities have called for the institution of routine melanoma screening recommendations, the United States Preventative Service Task Force (USPSTF) stopped short of endorsing annual screening WBEs as an effective prevention measure in 2016 due to insufficient evidence. Because of the paucity of data examining potential screening-related harms or program feasibility concerns in the United States, the USPSTF limited its support for WBE as a recommended intervention for patients at particularly high risk of cutaneous malignancy (10).

Despite the recommendations issued by the USPSTF, evidence does exist which supports the concept of widespread screening to facilitate early melanoma detection and decreased mortality (11). One of the most cited study examining the feasibility and efficacy of a population-based melanoma screening program is the SCREEN project in Northern Germany. Begun in 2003, this program involved the screening of over 360,000 patients by physicians of various specialties who had completed an 8 h WBE training course. The SCREEN project resulted in a 30% increase of melanoma detection within the study population and an approximately 50% decrease in melanoma-related mortality compared with the rest of Germany (12). Another study with similar findings was performed in Australia in 2008. This case-control study demonstrated a 38% increase in the probability of a thin melanoma (<0.75 mm) being identified and that pre-diagnosis WBE screening leads to a 14% risk reduction of thick melanoma (>0.75 mm) diagnosis (5).

Although broad consensus is lacking regarding routine melanoma screening in the United States, many dermatologists, oncologists, and primary care providers have incorporated annual WBEs into their practices and institutional preventative care programs. In addition, there is uniform agreement that patients at increased risk of melanoma should absolutely undergo yearly WBE, ideally at the hands of a dermatologist. Such patients include those with albinism, XP, a family history of melanoma, a personal history of skin cancer and individuals on chronic immunosuppressive medications (7).

Clinical Diagnosis

PATIENT HISTORY

Typically, melanoma lesions are incidentally discovered during routine skin examination (5). Occasionally, patients may be alerted to the presence of a concerning

TABLE 1 Fitzpatrick Classification of Skin Types I through VI

Type I	Type II	Type III	Type IV	Type V	Type VI
White skin. Always burns, never tans.	Fair skin. Always burns, tans with difficulty.	Average skin color. Sometimes mild burn, tan about average.	Light-brown skin. Rarely burns. Tans easily.	Brown skin. Never burns. Tans very easily.	Black skin. Heavily pigmented. Never burns, tans very easily.

nodule by persistent itching, bleeding, or crusting of a pigmented lesion. Unfortunately, most melanomas are asymptomatic and may only cause the aforementioned symptoms of local inflammation after growth progression has occurred (7). Once the diagnosis is suspected, questions pertaining to sites of potential metastasis should be included in the history. Potential indicators of metastatic spread may include seizures, headaches, vision changes, coughing, hemoptysis, shortness of breath, dyspnea, changes in bowel habits, new-onset back pain, or any systemic symptoms (fevers, chills, night sweats, weight loss, etc.). Other concerning items within a patient's history that should alert the examining physician include a past medical history of cutaneous malignancy, chronic sun exposure, history of blistering sun burns, use of tanning salons, family history of melanoma, pancreatic cancer, other familial syndromes, or a procedural history of prior skin biopsies. Finally, it should be noted that patients with fair skin (Fitzpatrick type I) are at increased risk of melanoma compared with those with darker skin (Fitzpatrick type VI) (Table 1) (13–15).

PHYSICAL EXAMINATION FINDINGS

During a clinical examination, any pigmented lesion with features contained within the "ABCDE" mnemonic should be considered suspicious for melanoma (Figure 1). Developed for both physicians and patients to recognize characteristics often associated with melanoma, the ABCDE system includes **A**symmetry, **B**order irregularity, **C**olor variegation, **D**iameter larger than 6 mm, and **E**volution or timing of the lesion's growth. Should such a lesion be identified, the surrounding area should be assessed for possible satellite lesions or in-transit metastatic foci (7). Once a concerning lesion is thoroughly assessed, the remaining cutaneous surfaces (i.e., scalp, perineum, interdigital space, genitalia, and subungual regions) should be closely inspected for the presence of any additional lesions of suspicion. All lesions with a benign appearance should be documented and all lymph node basins should be palpated for lymphadenopathy (14).

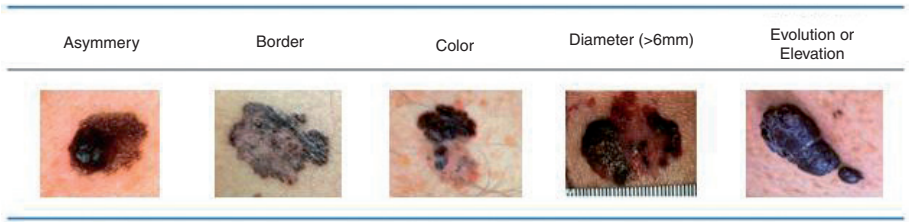

Figure 1 **ABCDE System for Diagnosis of Melanoma.**

Diagnostic Strategies

Once a suspicious lesion is assessed and properly documented, biopsy and histologic review should be considered. Sampling of the lesion in question can be performed through several methods, including excisional biopsy and partial biopsy. As previously discussed, vertical depth of invasion is among the most important prognostic factors in melanoma diagnosis. Thus, excisional biopsy of the entire specimen with narrow margins is the most effective way to facilitate proper diagnosis and treatment planning. This approach is supported by the American Academy of Dermatology and has long been preferred as the biopsy technique of choice by surgical oncologists involved in the definitive treatment of this disease process.

Alternatively, partial biopsy may be performed and is typically completed via a punch or shave technique. A punch biopsy, if properly positioned, may be advantageous since the provision of a full-thickness sample is possible (14). However, this technique often requires suture-based closure, which lengthens the encounter. Despite its frequent use among dermatologists and primary care physicians, partial biopsy performed via the shave technique has previously raised doubts regarding staging accuracy and histologic interpretation due to its ability to transect a segment of the lesion in question. Despite historical resistance, a properly performed shave biopsy is easy to execute, typically does not require cutaneous suturing, and can be quickly completed in a busy outpatient setting (14, 16). A recently published, multi-institutional, retrospective study of 600 patients challenged decades of surgical dogma. This study demonstrates that partial biopsy for melanoma does not adversely affect disease-free survival or overall survival and rarely results in the need for repeat biopsy. The authors conclude that partial biopsy is safe and should be performed by primary care providers and specialists alike. Therefore, it is reasonable to complete either excisional or partial biopsy when concerning lesions are encountered (16).

HISTOLOGIC CONFIRMATION

Diagnostic confirmation involves routine histologic analysis by the receiving pathology department (14). Microscopic findings including cytologic atypia, amplified cellularity, and the number of dermal mitotic figures should be noted in

an effort to distinguish benign disease from malignant melanoma. Established guidelines recommend the formal reporting of Breslow thickness (mm), histologic subtype; dermal mitotic rate; peripheral margin status; deep margin status; and the presence or absence of histologic ulceration, microsatellitosis, tumor infiltrating lymphocytes, cellular regression, angiolymphatic invasion, vertical growth phase, neurotropism, and pure desmoplasia. In addition, Clark's levels of anatomic staging should be reported for lesions <1 mm in thickness. By combining the reported histologic features with a patient's gross clinical findings, the proper diagnosis can be achieved and ambiguity avoided (13).

DISEASE TYPES AND PROGNOSTIC FACTORS

The primary histologic subtypes of melanoma include superficial spreading, lentigo maligna, nodular, acral lentiginous, desmoplastic, and amelanotic (Table 2) (17). *In situ* melanoma is considered Stage 0 and occurs when tumor cells are microscopically identified but have not penetrated the epidermis (18). Comprising approximately 70% of confirmed melanomas, the superficial spreading subtype is the most common type and arises from an existing nevus. The lentigo maligna subtype is less common, typically demonstrates slow progression, and frequently appears in sun-exposed areas (face, head, etc.). Nodular melanomas are characterized by the absence of a radial growth phase, variable presentation, and robust vertical invasion. Acral lentiginous melanomas have a higher incidence in patients with darker skin pigmentation and frequently occur on the palms, soles, and subungual spaces. Desmoplastic melanomas are uncommon lesions that are typically seen in elderly patients and feature limited spindle or atypical cells. Possibly the most challenging subtype in terms of diagnosis, amelanotic melanomas have a characteristic absence of pigmentation and are considered rare (14).

TABLE 2 Melanoma Subtypes

Subtype	Frequency	Characteristic
Superficial spreading	70%	Arises from existing nevus.
Nodular	5%	Absence of a radial growth phase, variable presentation, and robust vertical invasion.
Lentigo Maligna	4–15%	Typically demonstrates slow progression, and frequently appears in sun-exposed areas (i.e., face, head, etc.)
Acral lentiginous	5%	Has higher incidence in patients with darker skin pigmentation and frequently occur on the palms, soles, and subungual spaces.
Amelanotic	4%	Characteristic absence of pigmentation and are considered rare.
Desmoplastic	Less than 4%	Rare melanoma seen in older adults that is characterized by scant spindle cells and minimal cellular atypia.

Melanoma Staging

Initially, the proper staging of melanoma is the result of clinical assessment and histologic confirmation. The American Joint Committee on Cancer TNM system is used with resultant clinical and pathologic staging assignment (Table 3) (18). Once the index lesion has been histologically confirmed as melanoma, additional characteristics that contribute to the T (tumor) stage include overall tumor thickness, presence of ulceration, and the presence of mitosis in lesions <1 mm in thickness (T1)(14, 19).

N (nodal) stage is determined by the number of involved lymph nodes. As previously discussed, nodal status should be initially assessed at the time of preoperative clinical examination. If palpable lymphadenopathy is encountered, nodal status should be confirmed via ultrasound-guided fine needle aspiration. If no clinical evidence of nodal involvement is present preoperatively, sentinel lymph node biopsy (SLB) should be performed at the time of surgery for all

TABLE 3 Melanoma Staging

Stage	Classification	5-year survival
Stage 0	Tis: Melanoma *in situ*	>98%
Stage I (A/B)	T1a: <0.8 mm and nonulcerated T1b: ≥0.8 mm or <0.8 mm with ulceration T2a: >1.0–2.0 mm without ulceration	97–92%
Stage II (A, B, C)	T2b: >1.0–2.0 mm with ulceration T3a: >2.0–4.0 mm without ulceration T3b: >2.0–4.0 mm with ulceration T4a: >4.0 mm without ulceration T4b: >4.0 mm with ulceration	81–53%
Stage III (A, B, C, D)	N1a: 1 clinically occult (in SLN biopsy) N1b: 1 clinically detected N1c: Presence of in-transit, satellite, and/or microsatellite mets N2a: 2–3 clinically occult (in SLN biopsy) N2b: 2–3, at least 1 clinically detected N2c: 1 clinically occult or detected, with in-transit, satellite, and/or microsatellite mets N3a: 4 or more clinically occult (in SLN biopsy) N3b: 4 or more, at least 1 of which clinically detected, or presence of any number of matted nodes N3c: 2 or more clinically occult or clinically detected with in-transit, satellite, and/or microsatellite mets	78–40%

Table continued on following page

TABLE 3 Melanoma Staging

Stage	Classification	5-year survival
Stage IV	M1a: Distant metastasis to skin, soft tissue including muscle, and/or nonregional lymph nodes. LDH not recorded or unspecified M1a(0): LDH not elevated M1a(1): LDH elevated M1b: Distant metastasis to lung with or without M1a sites of disease. LDH not recorded or unspecified M1b(0): LDH not elevated M1b(1): LDH elevated M1c: Distant metastasis to non-CNS visceral sites with or without M1a or M1b sites of disease. LDH not recorded or unspecified M1c(0): LDH not elevated M1c(1): LDH elevated M1d: Distant metastasis to CNS with or without M1a, M1b, or M1c sites of disease. LDH not recorded or unspecified M1d(0): LDH not elevated M1d(1) LDH elevated	20–15%

Adapted from Gershenwald JE et al. AJCC cancer staging manual. 8th ed. Amin MB, editors. Chicago, IL: American Joint Committee on Cancer; 2017. p. 563.

lesions >1 mm in thickness. In addition, SLB should be considered for lesions between 0.76 and 1.0 mm thickness when high-risk features are present (lymphovascular invasion, high mitotic count, ulceration, etc.). Current guidelines do not recommend SLB for lesions ≤0.75 mm thick (20).

M (metastatic) stage is assigned based on the presence or absence of metastatic disease and, if present, is further classified by the location (skin, lymph nodes, viscera, lungs, or increased serum lactate dehydrogenase). Melanoma without nodal or distant metastases is classified as Stage I or Stage II, depending on the depth of vertical invasion. Stage III disease includes patients with either gross or microscopic lymph node metastasis and Stage IV disease includes patients with evidence of distant metastasis (13, 14, 19, 21).

Unlike other solid malignancies, the use of cross-sectional imaging and serum laboratory analysis to facilitate initial clinical staging is not routinely recommended outside of Stage IV disease (22). However, computed tomography (CT) (with or without positron emission tomography [PET]) and magnetic resonance imaging (MRI) should be considered for all patients with specific symptoms, Stage III disease, or even Stage II melanoma with high-risk features. In the setting of Stage IV melanoma, CT imaging of the chest, abdomen, and pelvis should be obtained, and a brain MRI can be considered (13, 21).

Conclusion

As discussed in the subsequent sections of this book, treatment options for advanced stages of cutaneous melanoma have significantly expanded in recent years. Although many of these new interventional approaches have injected much prognostic optimism into the field as a whole, it must be emphasized that delayed or deficient elements of the initial melanoma evaluation process can limit patient outcomes and increase disease-related mortality. Clinicians involved in the diagnosis or treatment of cutaneous melanoma must be familiar with the importance of available screening options, the key steps of clinical and histologic diagnosis, and the staging ramifications of disease discovery. Improvement in these areas will reduce disease incidence and progression, and may afford increased hope to patients afflicted with cutaneous melanoma.

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References

1. Shellenberger R, Nabhan M, Kakaraparthi S. Melanoma screening: A plan for improving early detection. Ann Med. 2016;48(3):142–8. http://dx.doi.org/10.3109/07853890.2016.1145795
2. Howlader N, Noone AM, Krapcho M, Miller D, Bishop K, Kosary CL, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (eds). SEER Cancer Statistics Review, 1975-2014, National Cancer Institute. Bethesda, MD, https://seer.cancer.gov/csr/1975_2014/, based on November 2016 SEER data submission, posted to the SEER web site, April 2017.
3. Perlis C, Herlyn M. Recent advances in melanoma biology. Oncologist. 2004;9(2):182–7. http://dx.doi.org/10.1634/theoncologist.9-2-182
4. Garibyan L, Fisher DE. How sunlight causes melanoma. Curr Oncol Rep. 2010;12(5):319–26. http://dx.doi.org/10.1007/s11912-010-0119-y
5. Aitken JF, Elwood M, Baade PD, Youl P, English D, *Clinical whole-body skin examination reduces the incidence of thick melanomas.* Int J Cancer, 2010. 126(2): p. 450–8.
6. Leachman SA, Carucci J, Kohlmann W, Banks KC, Asgari MM, Bergman W, et al., *Selection criteria for genetic assessment of patients with familial melanoma.* J Am Acad Dermatol, 2009. 61(4): p. 677.e1-14.
7. Psaty EL, Scope A, Halpern AC, Marghoob AA, *Defining the patient at high risk for melanoma.* Int J Dermatol, 2010. 49(4): p. 362–76.
8. Gumaste PV, Penn LA, Cymerman RM, Kirchhoff T, Polsky D, McLellan B, *Skin cancer risk in BRCA1/2 mutation carriers.* Br J Dermatol, 2015. 172(6): p. 1498–506.
9. Sondak VK, Glass LF, Geller AC. Risk-stratified screening for detection of melanoma. JAMA. 2015;313(6):616–17. http://dx.doi.org/10.1001/jama.2014.13813
10. Wernli KJ, Henrikson NB, Morrison CC, Nguyen M, Pocobelli G, Whitlock, EP, *U.S. Preventive Services Task Force Evidence Syntheses, formerly Systematic Evidence Reviews*, in *Screening for Skin Cancer in Adults: An Updated Systematic Evidence Review for the U.S. Preventive Services Task Force*. 2016, Agency for Healthcare Research and Quality (US): Rockville (MD).

11. Weinstock MA, Ferris LK, Saul MI, Geller AC, Risica PM, Siegel JA, et al., *Downstream consequences of melanoma screening in a community practice setting: First results.* Cancer, 2016. 122(20): p. 3152–3156.
12. Breitbart EW, Waldmann A, Nolte S, Capellaro M, Greinert R, Volkmer B, et al., *Systematic skin cancer screening in Northern Germany.* J Am Acad Dermatol, 2012. **66**(2): p. 201–11.
13. Coit DG, Andtbacka R, Anker CJ, Bichakjian, CK, Carson WE, Daud A, et al., *Melanoma, version 2.2013: featured updates to the NCCN guidelines.* J Natl Compr Canc Netw, 2013. 11(4): p. 395–407.
14. Kauffmann RM, Chen SL. Workup and staging of malignant melanoma. Surg Clin North Am. 2014;94(5):963–72, vii. http://dx.doi.org/10.1016/j.suc.2014.07.001
15. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. Arch Dermatol. 1988;124(6):869–71. http://dx.doi.org/10.1001/archderm.1988.01670060015008
16. Zager JS, Hochwald SN, Marzban SS, Francois R, Law KM, Davis AH, et al., *Shave biopsy is a safe and accurate method for the initial evaluation of melanoma.* J Am Coll Surg, 2011. 212(4): p. 454–60; discussion 460-2.
17. Markovic SN, Erickson LA, Rao RD, Weenig RH, Pockaj BA, Bardia A, et al., *Malignant melanoma in the 21st century, part 1: epidemiology, risk factors, screening, prevention, and diagnosis.* Mayo Clin Proc, 2007. 82(3): p. 364–80.
18. Amin MB, Edge SB, Greene FL, Byrd DR, Brookland RK, Washington MK, et al., editors. *AJCC Cancer staging manual.* 8th ed. New York: Springer; 2017.
19. Balch CM, Gershenwald JE, Soong SJ, Thompson JF, Atkins MB, Byrd DR, et al., *Final version of 2009 AJCC melanoma staging and classification.* J Clin Oncol, 2009. 27(36): p. 6199–206.
20. Coit DG, Thompson JA, Algazi A, Andtbacka R, Bichakjian CK, Carson WE, et al., *Melanoma, Version 2.2016, NCCN Clinical Practice Guidelines in Oncology.* J Natl Compr Canc Netw, 2016. 14(4): p. 450–73.
21. NCCN. NCCN clinical practice guidelines in oncology. Melanoma V. 1.2017. Fort Washington, PA: NCCN; 2016.
22. Pandalai PK, Dominguez FJ, Michaelson J, Tanabe KK, *Clinical value of radiographic staging in patients diagnosed with AJCC stage III melanoma.* Ann Surg Oncol, 2011. 18(2): p. 506–13.

7 Surgical Management of Melanoma

KENNETH M. JOYCE

Department of Plastic & Reconstructive Surgery, Galway University Hospital, Galway, Ireland

Author for correspondence: Kenneth M. Joyce, Department of Plastic & Reconstructive Surgery, Galway University Hospital, Galway, Ireland.
E-mail: kennethjoyce1@gmail.com

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Abstract This chapter discusses the surgical principles in the management of melanoma. Surgery remains the mainstay of treatment of primary melanoma, and in the majority of cases it is curative. Appropriate surgical management is critical for the diagnosis, staging, and optimal treatment of both *in situ* and invasive primary cutaneous melanoma. Surgical management is dependent on the stage of the disease, and therefore this chapter evaluates localized, regional, and metastatic disease. The concept of sentinel lymph node biopsy is discussed along with its benefits, pitfalls, and prognostic significance. Furthermore, several important surgical issues are discussed, including the extent of surgical margins, Mohs micrographic surgery for melanoma *in situ*, and the role of metastasectomy.

Key words: Lymphadenectomy; Lymph node; Melanoma; Sentinel node biopsy; Surgery

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Introduction

The incidence of melanoma is increasing worldwide, with most cases diagnosed at an early stage. However, unlike many other cancers, the mortality rate for melanoma remains stable largely as a result of decreasing mortality among younger individuals and increasing mortality among older individuals. Surgery remains the mainstay of treatment of primary melanoma, and in the majority of cases it is curative. Appropriate surgical management is critical for the diagnosis, staging, and optimal treatment of invasive primary cutaneous melanoma. The goals of surgery include histologic confirmation of the diagnosis, accurate microstaging, followed by appropriate excision of the margin around the primary site to minimize the risk of local recurrence. This chapter describes the surgical management of the primary site, the regional lymph node basin, as well as surgical options for distant disease.

Surgical Treatment of Localized Disease

Surgery remains the best option for cure in localized, invasive melanoma, with an overall 5-year survival rate of 92% (1). Wide local excision is the current standard of care for localized cutaneous melanoma. This wide excision contrasts to a narrow excision (1–2 mm) used to biopsy a lesion clinically suspicious for melanoma. A biopsy will provide the pathologist with a specimen that can then be examined to confirm the diagnosis of melanoma and determine the Breslow thickness. The margin required when carrying out a wide local excision is determined by the Breslow thickness. When carrying out a wide local excision, the excised specimen should extend down to the level of the underlying muscular fascia. Currently, there is no evidence to suggest that excising the underlying fascia leads to improved outcome (2).

REVIEW OF CURRENT GUIDELINES FOR EXCISION

Standards are well established for peripheral margins of excision (Table 1). These guidelines are based on data from randomized controlled trials. The excision margins are measured intraoperatively on the skin. Current guidelines for melanoma *in situ* recommend a 5 mm–1 cm peripheral margin. For large melanoma, *in situ* surgical margins >0.5 cm may be necessary to achieve histologically negative margins. A 0.5-cm margin for lentigo maligna melanoma *in situ* on the head and neck often results in an incomplete excision (4). This is often managed as a staged procedure, where histological clearance is confirmed prior to definitive reconstruction. A depth of excision that includes subcutaneous fat is generally sufficient for melanoma *in situ* (5).

MOHS MICROGRAPHIC SURGERY

Mohs micrographic surgery (MMS) is a surgical procedure which involves stepwise tangential excision of specimen margins up to normal-appearing skin,

TABLE 1 Recommended Margins for Surgical Excision

Tumor thickness	Recommended clinical margins[a]
In situ	0.5–1.0 cm
≤1.00 mm	1.0 cm
1.01–2.00 mm	1–2 cm
2.01–4.00 mm	2.0 cm
≥4.01 mm	2.0 cm

[a]Guidelines from National Comprehensive Cancer Network on Melanoma 2016 (3).

followed by immediate microscopic examination of the entire surgical margin. In contrast to surgical excision, MMS allows for the examination of 100% of the peripheral margins. Despite the advantages of MMS as a tissue-sparing procedure, controversy surrounds the use of frozen sections to identify malignant melanocytic cells (6). Pathological difficulties encountered include: vacuolated keratinocytes mimicking melanocytes, processing artifact, and dermal inflammatory cells that may obscure the melanocytes in frozen sections (7). MMS is a useful approach for clinically ill-defined lentigo maligna lesions; however, its use is not generally supported for invasive melanoma. Although the literature is controversial, enough studies exist with 5-year follow-up to suggest this approach is superior to traditional surgical excision in ill-defined lentigo maligna lesions (8). The central component of the tumor specimen should always be sent for permanent section assessment to rule out an invasive component. Mohs is not an acceptable modality for invasive melanoma.

Clinically Negative Regional Lymph Nodes

SENTINEL LYMPH NODE BIOPSY

Concept

Since its introduction in 1992, Sentinel Lymph Node Biopsy (SLNB) has become an established investigation in melanoma (9). Lymphatic mapping and SLNB is the standard approach for the management of patients with melanoma in whom there is a substantial risk of regional node metastasis. The concept behind lymphatic mapping is that sites of cutaneous melanoma have stepwise patterns of lymphatic spread and that one or more nodes are the first to be involved with metastatic disease within a given lymph node basin. If the sentinel lymph nodes are not involved, the entire basin should be free of tumor (10).

Indications

Sentinel node biopsy is indicated for melanomas ≥1.0 mm in Breslow thickness. There is no consensus regarding the application or clinical implications of SLNB in patients whose melanomas are <1 mm in thickness, and indications continue to evolve (Table 2). Based on available evidence, high-risk patients with melanomas between 0.75 and 1.00 mm in thickness may be appropriate candidates to be considered for SLN biopsy; however, there is little rationale in performing SLNB on the overwhelming majority of patients with melanomas <0.75 mm in thickness (14).

Sentinel node sensitivity and specificity

Morton et al. reported the sensitivity rate of SLNB as 95.3% overall: 99.3% for the groin, 95.3% for the axilla, and 84.5% for the neck basins (15). Reported rates of SN metastasis are 12 to 20% for 1- to 2-mm melanomas, 28 to 33% for 2- to 4-mm melanomas, and 28 to 44% for melanomas thicker than 4 mm (16). The rate of false-negative SLNB in thin melanomas was reported in a recent meta-analysis to be 12.5% (17). Current standard therapy for patients with a positive SLNB is completion dissection of all involved nodal basins. The recent DeCOG-SLT trial showed for patients with micrometastatic sentinel node disease (metastases <1 mm diameter), no survival benefit was present comparing nodal observation and completion lymphadenectomy (18).

TABLE 2 Current Guidelines for Performing SLNB

Guideline	Year
National Comprehensive Cancer Network Practice Guidelines (NCCN) (3): "In general, SLNB is not recommended for primary melanoma <0.75 mm thick. For melanomas 0.76–1.00 mm, SLNB may be considered in the appropriate clinical context"	2017
National Institute of Clinical Excellence (NICE) (11): "Do not offer imaging or sentinel lymph node biopsy to people who have stage IA melanoma or those who have stage IB melanoma with a Breslow thickness of 1 mm or less"	2015
American Society of Clinical Oncology and Society of Surgical Oncology Joint Clinical Practice Guideline (ASCO/SSO) (12): "Available evidence does not support routine SLN biopsy for patients with melanomas that are T1 or <1mm Breslow thickness although it may be considered in selected high-risk cases" Such high-risk factors may include Breslow thickness >0.75 mm, ulceration, or mitoses ≥1/mm^2	2012
European Society for Medical Oncology (ESMO) (13): "SLN biopsy should be performed for tumour thickness of >1mm and/or ulceration"	2012

Benefit

A positive SLNB is the best predictor of recurrence and survival in patients with clinically node negative cutaneous melanoma (19). Indications for performing SLNB is a balance between the likelihood of finding a positive SLN, the risk of the procedure, as well as the likely benefits that will accrue to the patient from the knowledge of their SLN status (14). It selects appropriate patients for completion lymph node dissection with potential for regional disease control. It also identifies a homogenous group of patients who may benefit from adjuvant therapy and enrollment in clinical trials.

Potential risks

Sentinel node biopsy carries significantly less risk of complications compared to lymph node dissection. Sentinel node biopsy has an overall complication rate of 5% (20). Potential complications include infection (1%), lymphedema (0.7%), hematoma/seroma (2%), and sensory nerve injury (0.2%) (20). Furthermore, there is a risk of incorrectly biopsying a node which is not the sentinel node for the primary site, that is, a false-negative sentinel node. This is relatively high in head and neck melanomas, with a false-positive rate of 18–29% reported in some studies (21).

The multicenter selective lymphadenectomy trial

The landmark Multicenter Selective Lymphadenectomy Trial-I (MSLT-I) is the largest trial comparing the use of SLNB and elective lymph node dissection (ELND) to observe patients with intermediate thickness melanomas in determining prognosis and its impact on survival (15, 22). Long-term data from the MSLT-I show improved 10-year disease-free survival but fail to show improved melanoma-specific survival (22). The MSLT-I reported their morbidity associated with SLNB to be 10.1%, with nearly half of these complications from seroma or hematoma, followed by infection (4.6%) and wound dehiscence (1.2%) (15). The recent MSLT-IIR trial showed that complete lymph node dissection, following a positive sentinel node biopsy, increased the rate of regional disease control and provided prognostic information but did not increase melanoma-specific survival (23).

Elective Lymph Node Dissection

There are two different approaches toward lymphadenectomy: prophylactic or ELND of the regional nodes draining the primary tumor versus delayed lymphadenectomy only when recurrences occur in the nodal basin (24). Opponents of ELND consider prophylactic excision of lymph nodes unnecessary because the incidence of histologically positive regional nodes at the time of resection of the primary melanoma in patients with clinical Stage I disease is only 20% (25).

Prior to the introduction of SLNB, ELND was advocated as an approach to the regional lymph nodes. However, the success of SLNB in predicting regional lymph node involvement has obviated a possible role for ELND. Multiple prospective randomized trials were conducted to evaluate the role of ELND, but these did not confirm a substantial survival benefit from ELND (26). ELND should not be considered in treating patients with melanoma.

Clinically Apparent Regional Lymph Nodes

THERAPEUTIC LYMPHADENECTOMY

Regional lymph node involvement can be diagnosed cytologically using either fine needle aspirate or image-guided biopsy. Therapeutic lymphadenectomy is the preferred treatment in patients with regional clinical lymph node involvement from melanoma (27). The 10-year survival rate in patients with metastatic involvement of regional lymph nodes is approximately 20–40% (28). The tumour burden within the regional lymph node is an important prognostic factor, with a high nodal involvement associated with a poorer outcome (28). Since melanoma has a high risk of involvement of multiple regional lymph nodes within a nodal basin, a complete regional lymphadenectomy, rather than partial dissection or sampling, is necessary (29). In the axillary basin, a complete dissection (levels I, II, and III) should be carried out. The role of a deep ilioinguinal dissection is controversial, since no survival benefit has been demonstrated with the addition of a more extensive dissection (30). Some surgeons choose to include a deep ilioinguinal dissection to the superficial inguinal node dissection when the highest superficial node (Cloquet's node) contains metastatic melanoma. However, this practice is disputed, and not standard of care (30).

SURGICAL CONSIDERATIONS

In the head and neck region, lymph nodes at risk for metastatic melanoma include the parotid, cervical (levels I through V), and post-auricular and occipital nodal basins. Typically, lesions in the face and anterior scalp drain to the parotid and cervical levels I–IV (31). Lesions in the posterior scalp drain to cervical levels II–V, and occipital and post-auricular basins (31). Most frequently, a functional neck dissection is performed, thereby preserving the internal jugular vein, sternocleidomastoid muscle, and the spinal accessory nerve.

Axillary lymphadenectomy should involve lymphatic clearing of levels I–III. This can be achieved through an S-shaped incision with attention during dissection to protect the axillary vein, and the long thoracic, thoracodorsal, and medial pectoral nerves.

Inguinal lymphadenectomy involves dissection of the superficial (inguinal) with or without the deep (ilioinguinal) nodes. Access is through a straight incision just below and parallel to the inguinal ligament, with extensions onto the abdomen laterally or down the thigh medially if needed. The superficial nodes lie within the femoral triangle (bounded by the adductor longus muscle medially, the sartorius muscle laterally, and the inguinal ligament superiorly).

A sartious muscle transposition to protect the femoral vessels is often carried out to protect against postoperative wound problems, especially if adjuvant radiotherapy may be necessary (32). More recently, minimally invasive inguinal lymphadenectomy can be carried out, which obviates the need for routine sartorius muscle transposition (33).

In Transit Metastatic Disease

In transit metastatic disease includes any skin or subcutaneous metastases that are more than 2 cm from the primary lesion but are not beyond the regional nodal basin (34). Satellite metastases are defined as lesions occurring within 2 cm of the primary tumor. In the absence of distant metastatic disease, surgical excision is the treatment of choice when feasible. Regional chemotherapy in the form of isolated limb perfusion (ILP) or isolated limb infusion (ILI) is reserved for unresectable recurrent disease. ILP allows higher concentrations of drugs to be administered to the affected limb without systemic toxic effects. This is done by surgically separating the inflow and outflow, of the affected limb, from the rest of the body (35). ILI involves percutaneously placed venous and arterial catheters to allow infusion of chemotherapy via an arterial catheter, and a pneumatic tourniquet is used proximally to isolate the extremity (36). ILI differs from ILP in that ILI circulates blood in an affected extremity at a much slower rate than ILP and for only 30 min, and hyperthermia is not achieved (35). There are no randomized controlled trials comparing ILP to ILI, but recent studies showed the overall response rate was higher with ILP than with ILI (79% in 294 patients vs. 64% in 313 patients), but ILP resulted in more grade 5 toxicity (37). If neither surgery nor regional chemotherapy is appropriate, radiation therapy may provide palliative benefit. Furthermore, talimogene laherparepvec (T-VEC) is an option to treat unresectable, injectable, cutaneous, dermal, subcutaneous, or nodal metastases for patients with limited visceral disease (38).

Surgical Treatment of Metastatic Melanoma

The introduction of effective systemic therapies (e.g., BRAF/MEK, Anti-CTLA4, and PD-1 inhibitors) for patients with metastatic melanoma has altered the approach to management of patients with metastatic disease (39). Surgical metastasectomy plays a role in carefully selected patients who have limited sites of metastatic disease, either at first presentation of metastatic disease or if they have had a high-quality response to immunotherapy or potentially molecularly targeted therapy (40).

For in transit or satellite metastases confined to skin and subcutaneous tissue, the most appropriate management is complete excision with a small margin (41). Although widespread metastatic disease usually develops in most cases, complete resection of metastatic disease is associated with prolonged survival in up to 40% of cases (42). Symptomatic, easily resected metastases are also appropriately resected in a palliative setting, even in patients with multiple other sites of disease.

Conclusion

Surgery remains the best option for cure in localized, invasive melanoma, with an overall 5-year survival rate of 92%. MMS is a useful approach for clinically ill-defined lentigo maligna lesions; however, its use is not generally supported for invasive melanoma. Sentinel node biopsy is indicated for melanomas ≥1 mm in Breslow thickness.

Therapeutic lymphadenectomy is the preferred treatment in patients with regional lymph node involvement, with a 10-year survival rate in approximately 20–40% of patients with metastatic involvement of regional lymph nodes

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References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65(2):87–108. http://dx.doi.org/10.3322/caac.21262
2. Hunger R, Seyed Jafari S, Angermeier S, Shafighi M. Excision of fascia in melanoma thicker than 2 mm: No evidence for improved clinical outcome. Br J Dermatol. 2014;171(6):1391–6. http://dx.doi.org/10.1111/bjd.13478
3. Kunishige JH, Brodland DG, Zitelli JA. Margins for standard excision of melanoma in situ. J Am Acad Dermatol. 2013;69(1):164. http://dx.doi.org/10.1016/j.jaad.2013.01.040
4. Ethun CG, Delman KA. The importance of surgical margins in melanoma. J Surg Oncol. 2016;113(3):339–45.
5. Dawn ME, Dawn AG, Miller SJ. Mohs surgery for the treatment of melanoma in situ: A review. Dermatol Surg. 2007;33(4):395–402. http://dx.doi.org/10.1097/00042728-200704000-00001
6. Bub JL, Berg D, Slee A, Odland PB. Management of lentigo maligna and lentigo maligna melanoma with staged excision: A 5-year follow-up. Arch Dermatol. 2004;140(5):552–8. http://dx.doi.org/10.1001/archderm.140.5.552
7. Erickson C, Miller SJ. Treatment options in melanoma in situ: Topical and radiation therapy, excision and Mohs surgery. Int J Dermatol. 2010;49(5):482–91. http://dx.doi.org/10.1111/j.1365-4632.2010.04423.x
8. Morton DL, Wen D-R, Wong JH, Economou JS, Cagle LA, Storm FK, et al. Technical details of intraoperative lymphatic mapping for early stage melanoma. Arch Surg. 1992;127(4):392–9. http://dx.doi.org/10.1001/archsurg.1992.01420040034005
9. Reintgen D, Cruse CW, Wells K, Berman C, Fenske N, Glass F, et al. The orderly progression of melanoma nodal metastases. Ann Surg. 1994;220(6):759. http://dx.doi.org/10.1097/00000658-199412000-00009
10. Joyce K, McInerney N, Joyce C, Jones D, Hussey A, Donnellan P, et al. A review of sentinel lymph node biopsy for thin melanoma. Ir J Med Sci. 2015;184(1):119–23. http://dx.doi.org/10.1007/s11845-014-1221-1
11. Morton D, Cochran A, Thompson J, Elashoff R, Essner R, Glass E, et al. Multicenter Selective Lymphadenectomy Trial Group: Sentinel node biopsy for early-stage melanoma: Accuracy and

morbidity in MSLT-I, an international multicenter trial. Ann Surg. 2005;242(3):302–11. http://dx.doi.org/10.1097/01.sla.0000181092.50141.fa
12. Rousseau DL, Ross MI, Johnson MM, Prieto VG, Lee JE, Mansfield PF, et al. Revised American Joint Committee on Cancer staging criteria accurately predict sentinel lymph node positivity in clinically node-negative melanoma patients. Ann Surg Oncol. 2003;10(5):569–74. http://dx.doi.org/10.1245/ASO.2003.09.016
13. Valsecchi ME, Silbermins D, de Rosa N, Wong SL, Lyman GH. Lymphatic mapping and sentinel lymph node biopsy in patients with melanoma: A meta-analysis. J Clin Oncol. 2011;29(11):1479–87. http://dx.doi.org/10.1200/JCO.2010.33.1884
14. Leiter U, Stadler R, Mauch C, Hohenberger W, Brockmeyer N, Berking C, et al. Complete lymph node dissection versus no dissection in patients with sentinel lymph node biopsy positive melanoma (DeCOG-SLT): A multicentre, randomised, phase 3 trial. Lancet Oncol. 2016;17(6):757–67. http://dx.doi.org/10.1016/S1470-2045(16)00141-8
15. Wagner JD, Ranieri J, Evdokimow DZ, Logan T, Chuang T-Y, Johnson CS, et al. Patterns of initial recurrence and prognosis after sentinel lymph node biopsy and selective lymphadenectomy for melanoma. Plast Reconstr Surg. 2003;112(2):486–97. http://dx.doi.org/10.1097/01.PRS.0000070989.23469.1F
16. Wrightson WR, Wong SL, Edwards MJ, Chao C, Reintgen DS, Ross MI, et al. Complications associated with sentinel lymph node biopsy for melanoma. Ann Surg Oncol. 2003;10(6):676–80. http://dx.doi.org/10.1245/ASO.2003.10.001
17. Leiter U, Eigentler TK, Häfner H-M, Krimmel M, Uslu U, Keim U, et al. Sentinel lymph node dissection in head and neck melanoma has prognostic impact on disease-free and overall survival. Ann Surg Oncol. 2015;22(12):4073–80. http://dx.doi.org/10.1245/s10434-015-4439-x
18. Morton DL, Thompson JF, Cochran AJ, Mozzillo N, Nieweg OE, Roses DF, et al. Final trial report of sentinel-node biopsy versus nodal observation in melanoma. N Engl J Med. 2014;370(7):599–609. http://dx.doi.org/10.1056/NEJMoa1310460
19. Faries MB, Thompson JF, Cochran AJ, Andtbacka RH, Mozzillo N, Zager JS, et al. Completion dissection or observation for sentinel-node metastasis in melanoma. N Engl J Med. 2017;376(23):2211–22. http://dx.doi.org/10.1056/NEJMoa1613210
20. Lens MB, Dawes M, Goodacre T, Newton-Bishop JA. Elective lymph node dissection in patients with melanoma: Systematic review and meta-analysis of randomized controlled trials. Arch Surg. 2002;137(4):458–61. http://dx.doi.org/10.1001/archsurg.137.4.458
21. Hein D, Moy R. Elective lymph node dissection in stage I malignant melanoma: A meta-analysis. Melanoma Res. 1992;2(4):273–8. http://dx.doi.org/10.1097/00008390-199211000-00008
22. Balch CM, Soong S-J, Ross MI, Urist MM, Karakousis CP, Temple WJ, et al. Long-term results of a multi-institutional randomized trial comparing prognostic factors and surgical results for intermediate thickness melanomas (1.0 to 4.0 mm). Ann Surg Oncol. 2000;7(2):87–97. http://dx.doi.org/10.1007/s10434-000-0087-9
23. Voit CA, van Akkooi AC, Schäfer-Hesterberg G, Schoengen A, Schmitz PI, Sterry W, et al. Rotterdam criteria for sentinel node (SN) tumor burden and the accuracy of ultrasound (US)-guided fine-needle aspiration cytology (FNAC): Can US-guided FNAC replace SN staging in patients with melanoma? J Clin Oncol. 2009;27(30):4994–5000. http://dx.doi.org/10.1200/JCO.2008.19.0033
24. Balch CM, Soong S-J, Gershenwald JE, Thompson JF, Reintgen DS, Cascinelli N, et al. Prognostic factors analysis of 17,600 melanoma patients: Validation of the American Joint Committee on Cancer melanoma staging system. J Clin Oncol. 2001;19(16):3622–34. http://dx.doi.org/10.1200/JCO.2001.19.16.3622
25. Rossi C, Mozzillo N, Maurichi A, Pasquali S, Quaglino P, Borgognoni L, et al. The number of excised lymph nodes is associated with survival of melanoma patients with lymph node metastasis. Ann Oncol. 2014;25(1):240–6. http://dx.doi.org/10.1093/annonc/mdt510
26. Badgwell B, Xing Y, Gershenwald JE, Lee JE, Mansfield PF, Ross MI, et al. Pelvic lymph node dissection is beneficial in subsets of patients with node-positive melanoma. Ann Surg Oncol. 2007;14(10):2867–75. http://dx.doi.org/10.1245/s10434-007-9512-7
27. Klop WMC, Veenstra HJ, Vermeeren L, Nieweg OE, Balm AJ, Lohuis PJ. Assessment of lymphatic drainage patterns and implications for the extent of neck dissection in head and neck melanoma patients. J Surg Oncol. 2011;103(8):756–60. http://dx.doi.org/10.1002/jso.21865

28. Bartlett EK, Meise C, Bansal N, Fischer JP, Low DW, Czerniecki BJ, et al. Sartorius transposition during inguinal lymphadenectomy for melanoma. J Surg Res. 2013;184(1):209–15. http://dx.doi.org/10.1016/j.jss.2013.04.033
29. Delman KA, Kooby DA, Ogan K, Hsiao W, Master V. Feasibility of a novel approach to inguinal lymphadenectomy: Minimally invasive groin dissection for melanoma. Ann Surg Oncol. 2010;17(3):731–7. http://dx.doi.org/10.1245/s10434-009-0816-7
30. Balch CM, Gershenwald JE, Soong S-J, Thompson JF, Atkins MB, Byrd DR, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol. 2009;27(36):6199–206. http://dx.doi.org/10.1200/JCO.2009.23.4799
31. Eggermont AM, de Wilt JH, ten Hagen TL. Current uses of isolated limb perfusion in the clinic and a model system for new strategies. Lancet Oncol. 2003;4(7):429–37. http://dx.doi.org/10.1016/S1470-2045(03)01141-0
32. Beasley GM, Petersen RP, Yoo J, McMahon N, Aloia T, Petros W, et al. Isolated limb infusion for in-transit malignant melanoma of the extremity: A well-tolerated but less effective alternative to hyperthermic isolated limb perfusion. Ann Surg Oncol. 2008;15(8):2195–205. http://dx.doi.org/10.1245/s10434-008-9988-9
33. Beasley GM, Caudle A, Petersen RP, McMahon NS, Padussis J, Mosca PJ, et al. A multi-institutional experience of isolated limb infusion: Defining response and toxicity in the US. J Am Coll Surg. 2009;208(5):706–15. http://dx.doi.org/10.1016/j.jamcollsurg.2008.12.019
34. Sloot S, Rashid OM, Zager JS. Intralesional therapy for metastatic melanoma. Exp Opin Pharmacother. 2014;15(18):2629–39. http://dx.doi.org/10.1517/14656566.2014.967682
35. Leung AM, Hari DM, Morton DL. Surgery for distant melanoma metastasis. Cancer J. 2012;18(2):176. http://dx.doi.org/10.1097/PPO.0b013e31824bc981
36. Ollila DW. Complete metastasectomy in patients with stage IV metastatic melanoma. Lancet Oncol. 2006;7(11):919–24. http://dx.doi.org/10.1016/S1470-2045(06)70938-X
37. Testori A, Faries MB, Thompson JF, Pennacchioli E, Deroose JP, Van Geel AN, et al. Local and intralesional therapy of in-transit melanoma metastases. J Surg Oncol. 2011;104(4):391–6. http://dx.doi.org/10.1002/jso.22029
38. Sosman JA, Moon J, Tuthill RJ, Warneke JA, Vetto JT, Redman BG, et al. A phase 2 trial of complete resection for stage IV melanoma. Cancer. 2011;117(20):4740–6. http://dx.doi.org/10.1002/cncr.26111
39. Coit DG, Thompson JA, Algazi A, Andtbacka R, Bichakjian CK, Carson WE, et al. NCCN guidelines insights: Melanoma, version 3.2016. J Natl Compr Cancer Netw. 2016;14(8):945–58. http://dx.doi.org/10.6004/jnccn.2016.0101
40. National Institute for Health and Care Excellence. Melanoma: Assessment and management: NICE guideline. 2015. Available from: https://www.nice.org.uk/guidance/ng14?unlid=942848662015114152954
41. Gershenwald JE, editor. Evidence-based clinical practice guidelines on the use of sentinel lymph node biopsy in melanoma. Am Soc Clin Oncol. 2013;33:e320. http://dx.doi.org/10.1200/EdBook_AM.2013.33.e320
42. Dummer R, Hauschild A, Guggenheim M, Keilholz U, Pentheroudakis G. Cutaneous melanoma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2012;23(Suppl 7):vii86–91. http://dx.doi.org/10.1093/annonc/mds229

8 Radiation Therapy for Melanoma

WENYIN SHI

Department of Radiation Oncology, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA 19107, USA

Author for correspondence: Wenyin Shi, Department of Radiation Oncology, Sidney Kimmel Medical College, Thomas Jefferson University, 111 S 11 ST, Suite G301, Philadelphia, PA 19107, USA. E-mail: Wenyin.shi@jefferson.edu

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Abstract: Although melanoma is a relative radioresistant tumor, radiation therapy (RT) remains a valid and effective treatment option for the management of melanoma. RT as a primary treatment is often offered in well-defined situations, such as medical inoperability, lentiginous melanoma, mucosal melanoma, and ocular melanoma. Adjuvant RT following lymphadenectomy in node-positive melanoma patients prevents local and regional recurrence; however, the role of adjuvant RT remains controversial and underutilized due to lack of overall survival benefit. On the other hand, RT is highly effective in providing symptom palliation for metastatic melanoma and is widely used. Advanced RT technologies such as stereotactic radiosurgery (SRS) and stereotactic body radiotherapy (SBRT) can achieve excellent local control with minimum toxicities. They are commonly used in the management of brain, lung, spine, and liver metastases. Most recently, it is under active investigation on combining RT with new systemic options, such as targeted therapy, or immunotherapy. The advancements in the treatment of patients with melanoma highlight the importance of multidisciplinary management in this disease. Radiation therapy will continue to be one of the key therapeutic options.

Key words: Melanoma; Radiation treatment; Stereotactic body radiation treatment; Stereotactic Radiosurgery

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Introduction

In the United States, in 2016, there were 76,380 new cases of melanoma and 10,130 deaths (1), and the incidence and mortality have been steadily increasing over the past decades (1, 2). Surgery remains the mainstay of treatment for most patients, particularly for patients with early stage disease. Radiation therapy, on the other hand, plays an active role in the management of patients with advanced stages of the disease. Definitive radiation therapy is suited for certain well-defined situations, including medical inoperability, lentigo maligna melanoma (LMM), mucosal melanoma, and ocular melanoma. For patients with node-positive disease, adjuvant radiation therapy (RT) following lymphadenectomy effectively prevents local and regional recurrence. For patients with advanced stage and metastatic disease, RT is highly effective in providing symptom palliation. Radiation therapy also plays a role in conjunction with systemic therapy, such as BRAF inhibitor, or immune therapy to achieve additive or even synergistic benefit. The comprehensive management of patients with melanoma warrants a multidisciplinary approach. Radiation therapy will continue to be one of the key therapeutic options.

Historical Perspective

RT works by inducing DNA damage in cancer cells. Historically, melanoma had been deemed a radioresistant tumor. This notion is derived from *in vitro* clonogenic cell death assay demonstrating a broad shoulder. Based on linear quadratic model, the broad shoulder in cell survival curves indicates high repair efficacy at low dose. The high repair capacity of melanoma cells is due to efficient enzymatic system, high proliferation capacity, poor cell differentiation, hypoxia-induced radioresistant stem cells, and abnormal apoptosis due to p53 functional attenuation (3–5). This broad shoulder in cell survival curve also indicates an increased sensitivity to higher dose per fraction (6, 7). Conflicting initial clinical experience with varying doses per fraction prompted a multicenter randomized Phase III study through the Radiation Therapy Oncology Group (RTOG). This study (RTOG 8305) directly compared two dose schemes. In this trial, 137 patients with measurable metastatic melanoma were randomized to 32 Gy in 8 Gy per fraction weekly versus 50 Gy in 2.5 Gy daily fractions. No difference in clinical response rate was observed (8). There have been multiple additional retrospective studies evaluating various hypofractionated regimens, which showed similar outcomes in all the regimens (9–12). Nonetheless, hypofractionated radiation with 2.5 Gy or higher per fraction has become commonplace in the treatment of melanoma given its tolerability, convenience, and low risk of late effects.

On the other hand, there have been significant advancements in RT with evolution of imaging techniques, such as high-resolution computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), as well as advances in radiation delivery techniques. Two-dimensional techniques

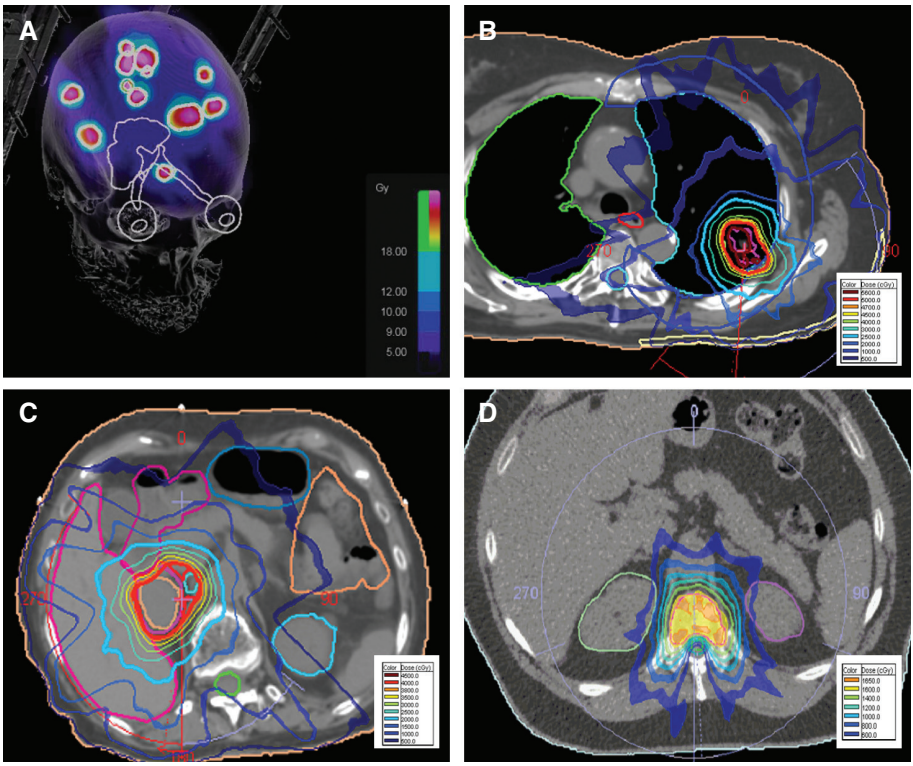

Figure 1 **Examples of SRS and SBRT treatment plans for melanoma metastasis: A) stereotactic radiosurgery (SRS) for multiple brain metastases; B) stereotactic body radiotherapy (SBRT) for lung metastasis; C) SBRT for adenral metastasis; and D) SBRT for spine metastasis.**

evolved to three-dimensional techniques with implementation of CT planning scans. The development of inverse planning such as intensity-modulated radiotherapy (IMRT) and volumetric-modulated arc therapy (VMAT) have allowed for even more precise RT delivery while sparing normal tissues and decreasing associated toxicity (13). High precision with patient immobilization, imaging guidance, and steep dose gradient allows for high-dose treatment delivery, which is most suitable for melanoma. Stereotactic radiosurgery (SRS) and stereotactic body radiation therapy (SBRT) are two examples of high-dose radiation therapy with high precision delivery (Figure 1). SRS refers to a precisely delivered single large dose of radiation achieved by multiple noncoplanar beams converging on a radiographically defined target (14). For this type of RT delivery, there is a steep decline of radiation dose just outside the target volume, thereby limiting the dose to normal critical structures. It is commonly used for treating melanoma brain metastasis. SBRT refers to high dose per fraction precise RT over approximately 2–5 treatment sessions. This dose fractionation scheme is particularly useful for patients with oligometastatic disease, such as lung, bone, liver, or adrenal metastasis.

Definitive RT for Lentiginous Patients

Lentigo maligna (LM) is the most common melanocytic malignancy of the head and neck. It has the potential for dermal invasion and development into invasive LMM (15). LM and LMM have slow growth rates and are associated with less potential for metastatic disease. While surgery is generally the treatment of choice for these lesions, the population most frequently affected are elderly patients who may not be optimal surgical candidates (16). To confound this, surgical option may also be limited due to the location and size of the lesion. Definitive RT has been used as a primary treatment modality for these patients with good long-term local control with acceptable cosmetic and functional outcomes (17–21). A recently published pooled analysis of eight studies with 349 patients with LM treated with definitive RT showed a 5% local recurrence rate (22). A majority of the patients who recurred were successfully salvaged with further RT, surgery, or other treatments. Another analysis of 454 patients from 10 studies demonstrated a mean recurrence rate of 11.5%, with the majority of studies having follow-up of more than 20 months (23). The side effects of radiation treatment are commonly mild, including pigment change, telangiectasia, and erythema (22). Definitive radiation therapy is a safe, well-tolerated, and effective treatment for LM and LMM.

RT in Mucosal Melanomas

Mucosal melanomas are rare, as compared to cutaneous melanomas. Primary sites of origin include the head and neck, anorectum, and vulvovaginal regions. It is uniquely different from cutaneous melanoma with respect to epidemiology, etiology, pathogenesis, and prognosis (24). They are clinically aggressive; even with aggressive surgical interventions, local recurrence can occur in 29–79% of patients (25–27). Therefore, adjuvant treatment, in particular radiation therapy, has been investigated with mixed results. A majority of the data pertains to head and neck mucosal melanomas and the addition of postoperative RT offers a local control benefit. The local recurrence rates with adjuvant radiation ranges from 15 to 30% (27–29). Despite the local control benefit of adjuvant radiation, there is no impact on overall survival, likely due to the high risk of systemic relapse (28–33).

Many patients present with unresectable lesions due to location and proximity to critical structures, particularly in the head and neck. Definitive radiation has been investigated in such setting. In a retrospective series of 28 patients with mucosal melanoma of the nasal cavity and paranasal sinuses, definitive RT was given to a dose of 50–55 Gy in 15–16 fractions and initial complete regression was observed in 22 out of 28 patients (79%). Local control of 49% at 3 years was observed in these patients (31). A similar report on 31 patients from multiple institutions treated with definitive RT showed a local control of 58.1% (33). The authors also noted that there was an increase in the local control and survival in patients who received a hypofractionated regimen with a dose per fraction greater than 3 Gy (33). Based on these findings, patients with unresectable mucosal melanoma, primary RT should be attempted for patients with localized disease.

Definitive RT for Ocular Melanomas

Ocular melanoma is a rare but potentially devastating malignancy arising from the melanocytes of the uveal tract, conjunctiva, or orbit; it represents less than 5% of all melanoma cases in the United States (34). Historically, enucleation of the eye has been the definitive treatment for patients with ocular melanoma. Over the past several decades, RT has become a crucial part of the successful treatment of ocular melanoma while preserving the eye and vision. Local control is exceptionally good with RT delivered by either external beam radiation therapy (EBRT) or episcleral plaque brachytherapy (35).

Brachytherapy has been used to treat intraocular tumors since 1930 (36). The custom-designed plaque is temporarily sutured to the sclera overlying the tumor. The plaque remains in place for 2–5 days, depending on the type of radioactive source. Preliminary experiences of episcleral brachytherapy used the high-energy isotope, cobalt-60 (^{60}Co) (37). Currently, iodine-125 (^{125}I) is the most commonly used isotope, but other low-energy isotopes, such as iridium-192, cesium-131, protactinium-103, and ruthenium-106/rhodium-106, have also been used (ABS-OOTF 2014) (38). The Collaborative Ocular Melanoma Study established the role of plaque brachytherapy in the management of ocular melanoma (39). This is a 12-year study that demonstrated relative equivalence of ^{125}I plaque (85 Gy) compared with enucleation in the prevention of metastatic melanoma for medium-sized choroidal melanoma. Plaque brachytherapy was effective in sterilizing the gross tumor, with local control being achieved in approximately 90% of patients. Only 5% of the patients require enucleation due to radiation-induced toxicities (39). Radiation-induced ocular injury is dose dependent and therefore lower doses have also been investigated to reduce toxicity. Doses as low as 69 Gy are capable of achieving similar rates of local control, distant metastasis-free survival, and overall survival as compared with 85 Gy (40). Specific dose constraints for tumors close to the macula have been suggested in order to minimize the potential of visual acuity loss. For such tumors, a dose less than 70 Gy to the tumor apex should be considered (41).

In terms of EBRT, proton therapy is most commonly used for the treatment of ocular melanoma. Compared to plaque therapy, proton therapy has advantages in treating larger tumors. A large, single institution study comparing proton beam with enucleation showed no apparent difference in long-term survival (42, 43). Favorable 5-year and 10-year local failure rates of 3.2% and 4.3%, respectively, were observed (43). For uveal melanoma, doses of 60 Gy delivered in four daily fractions of 15 Gy have been highly effective (44). Based on an analysis of 2069 patients treated at Harvard Cyclotron Laboratory and Proton Therapy Center at Massachusetts General Hospital between 1975 and 1997, a 15-year local control rate is 95% and the rate of eye preservation is 84%. A meta-analysis of 8809 patients with uveal melanoma included 7457 patients treated with charged particle therapy and 1352 patients with brachytherapy or enucleation. The rate of local recurrence was significantly lower with charged particle therapy than with brachytherapy (odds ratio 0.22) (45). However, there was no advantage with respect to mortality or enucleation when comparing particle therapy and brachytherapy (45). Dose reduction may be important for toxicity reduction in particle therapy as it is in brachytherapy, and a prospective randomized trial of lower-dose

(50 Gy) versus standard dose (70 Gy) proton radiation for small-to-moderate sized uveal melanoma showed no differences in a 5-year local or systemic recurrence or visual acuity loss, suggesting lower dose may be acceptable moving forward (44). In the past decade or two, linear accelerator (LINAC) stereotactic RT (SRT), or SRS with either LINAC or gamma knife has been investigated for its potential as an alternative option to proton beam (46–53). The initial experiences showed that SRT and SRS offer a noninvasive alternative to enucleation and brachytherapy in the management of uveal melanoma, with similar outcome to proton beam therapy (46–53).

Adjuvant RT for Cutaneous Melanomas

The role of RT in patients following surgical excision of cutaneous melanoma is multifaceted. With respect to adjuvant RT to the primary lesion, this is typically offered to patients who are at high risk for recurrence. Adjuvant RT to the primary site plays a role in the management of patients with desmoplastic neurotropic melanoma (DNM) as well as patients with lesions of the head and neck. Other indications for adjuvant radiation include tumor thickness >4 mm, ulceration, satellitosis, positive surgical margins, and mucosal origin (54).

There is a long history of adjuvant radiation after surgery to reduce local recurrence rate. The initial experience dated back to 1950s when patients thought to be at high risk of relapse were treated with brachytherapy or orthovoltage x-rays to the primary site (55). Subsequently, multiple retrospective studies further defined the role of adjuvant radiation. In 1981, Princess Margaret Hospital published a retrospective experience with 37 patients who underwent surgical resection of head and neck melanoma followed by adjuvant RT (56). This study provided an insight into the importance of radiation dose fractionation, as they found patients who received fractions greater than 4 Gy had improved local control (71% vs. 25%). A report from Sydney Melanoma Unit suggested that there may be an advantage in local control in patients with microscopically positive margins and/or adverse pathologic features who were offered postoperative RT (57). RT was delivered in a hypofractionated fashion to a total dose of 30–36 Gy in 5–7 doses over 2.5 weeks. The recurrence rate at 6 months was 11% in this cohort of 174 patients; this was compared with surgical data from the same time period which suggested that RT may have superior local control. However, there is no overall survival benefit due to high rate of distant failure (57).

With respect to patients with desmoplastic or neurotropic histology, data suggest that RT may offer a significant local control benefit. A retrospective analysis from Moffitt Cancer Center examined 277 patients with nonmetastatic desmoplastic melanoma who were treated with surgery with and without RT (58). At a median follow-up of 43.1 months, RT was associated with improved local control (HR, 0.15; 95% confidence interval, 0.06–0.39 [$P < 0.001$]), and this was particularly evident in patients with negative pathologic features (such as Breslow depth >4 mm, perineural invasion, or positive resection margins). Additional prospective data are needed to further clarify the role of adjuvant RT in desmoplastic or high-risk melanoma patients.

The role of adjuvant RT to the primary site in patients with a completely resected melanoma with neurotropic features is the question of a current clinical trial being run by Trans-Tasman Radiation Oncology Group (TROG) (www.ClinicalTrials.gov, NCT00975520). This is a 2-arm, randomized controlled trial in which patients are treated with surgical excision alone or surgical excision followed by adjuvant radiation to a dose of 48 Gy in 20 fractions over 4 weeks. The primary outcome of this trial is time to local relapse with the hypothesis that RT will improve local control in this select patient cohort.

Adjuvant RT for Regional Nodal Metastases

Adjuvant radiation after surgery decreases the risk of local recurrence for patients at high risk of regional failure after lymph node dissection. The high-risk factors include multiple positive nodes, large clinically palpable lymph nodes, extracapsular extension, and recurrence after prior lymph node dissection (54, 59–63). The largest retrospective analysis was performed by Agrawal et al. in which 615 patients who met the "high-risk" criteria for nodal relapse were offered adjuvant RT (60). The 5-year local recurrence rate was 10% in patients who received adjuvant radiation versus 41% in those patients who did not receive RT ($P < 0.0001$). High level of evidence was provided by Phase III trial run by the Australia and New Zealand Melanoma Trials Group and Trans-Tasman Radiation Oncology Group. In this trial, 250 patients with positive nodes who were deemed high risk were randomized following surgery to RT (48 Gy in 20 fractions) or observation. The criteria established for increased risk of regional recurrence were as follows: extracapsular extension, multiple positive nodes (>1 for parotid, >2 for neck and axilla, and >3 for groin location), and large lymph node (>3 cm for parotid, neck, and axilla, and >4 cm for groin location). After a mean follow-up of 73 months, lymph node recurrence in the RT arm was significantly lower as compared with observation (18% vs. 33%), but no benefit was observed with respect to relapse-free survival or overall survival (64).

Role of Palliative RT for Melanomas

Radiation therapy is highly effective for symptom palliation for melanoma distant metastasis. Common indications for palliative RT include pain, mass effect, tumor-related hemorrhage, and local irritation from skin or subcutaneous lesions (65). New RT techniques, such as SRS and SBRT, can achieve high probability of local control with very limited toxicity. SRS and SBRT are also preferred due to the relatively radioresistant nature of melanoma, and as a result improved efficacy can be achieved with higher dose per fraction. Ablative doses of RT such as those used in SBRT or SRS can be quite effective in the treatment of patients with limited number of metastases, or oligometastasis (66). Observed 5-year survival in patients with resectable metastases can be as high as 15 to 41% in the setting of few sites of distant metastases (67–70). In two series of patients from the University of Rochester, patients with 1–5 metastases (mainly breast, lung, and colon primary)

were treated with SBRT and the local control rate was reported to be 77% at 2 years (71). Duke University reported on a similar protocol and demonstrated a 2-year local control rate of 52.7% (72). SBRT for oligometastatic disease is a reasonable consideration for melanoma patients. There are currently eight open clinical trials investigating the use of SBRT in metastatic melanoma, most of which use a combination of an immune checkpoint inhibitor (www.ClinicalTrials.gov). This area of study is expected to significantly evolve in the coming decade.

Melanoma is the malignancy with the highest rate of brain metastasis, which occurs in more than 50% of patients with advanced melanoma (73). Intracranial disease progression is the cause of death in 20–54% of patients with disseminated melanoma (74). Despite advances in systemic therapy and surgical and radiation techniques, the prognosis of patients with brain metastasis remains poor. The median survival of these patients is 4.4 months and the 5-year survival rate is approximately 3% (75). Overall survival may be extended by effective locoregional treatment. Surgery, whole brain radiation therapy (WBRT), and SRS are all used in the treatment of brain metastasis; nonetheless, the best treatment remains controversial and many patients receive more than one modality (76, 77). Historically, WBRT is the *de facto* treatment for brain metastases. It can improve intracranial disease control and delay neurological decline (78). The most commonly prescribed dose schedule is 30 Gy in 10 fractions. Melanoma is considered a less radiosensitive tumor, and the local control with WBRT is poor. The estimated local control rate with WBRT at 6 months and 12 months are 37 and 15%, respectively (79), and the overall survival is unsatisfactory at 2–5 months (80). Besides dismal prognosis, WBRT is also associated with significant side effects, particularly high risk of neurocognitive decline (81, 82). Recently, there has been a paradigm shift toward more focused radiation treatment. For patients with limited brain metastases, SRS can be used as an alternative to WBRT without compromising overall survival, and with reduced neurocognitive impairment (83–86). Due to better response of melanoma to large radiation fraction dose, SRS treatment significantly improved the local control rate of melanoma brain metastases compared to those that were treated with WBRT (87, 88). The 12-month local control rate with SRS is about 65% (85–88). More impressively, SRS also contributes to improved overall survival from 4 months to 6–8 months as compared to WBRT (85, 89, 90). As a result, SRS alone should be considered the standard of care for patients with limited brain metastases (up to 10 brain metastases) and size suitable for SRS (usually ≤4 cm in diameter). Evaluation is ongoing as to whether the maximum number of lesions can be safely and effectively treated with SRS alone (91–93).

Bone metastases are common in patients with advanced melanoma. Bone metastases are important causes of morbidity and mortality in clinical practice and impair quality of life by causing pain, pathological fracture, spinal cord compression, bone marrow failure, and severe hypercalcemia. Approximately, 70% of bone metastases involve vertebrates, with thoracic and lumbar levels being the most common involvement sites. EBRT is a well-established treatment for vertebral metastases. Multiple prospective studies showed a pain response rate of 50–90% (94–98). RT achieves improvement in pain control in more than 65% of cases and re-calcification is observed in the areas with bone destruction on radiographs obtained a few months after treatment. There is no consensus on dose and fraction of palliative RT and many studies have been conducted to compare total dose and fraction (e.g., 8 Gy times 1, 10 Gy times 3, or 5 Gy times 4).

No difference was detected between longer and shorter therapies in any of the randomized studies including larger series (97, 99). As a result, 8 Gy in single-fraction RT was suggested as the standard of care for the palliation of uncomplicated painful bone metastases in the recent American Society for Radiation Oncology (ASTRO) guidelines (98, 100). However, conventional RT is limited by the low tolerance of the spinal cord and cauda equina, leading to subtherapeutic dose delivery for tumor control, particularly for melanoma. Local control for bone and/or spine metastasis treated with SRS and/or SBRT is also very favorable (70–90%) (101–106). SBRT treatment also has the advantage of better and more durable pain control for bone metastasis. A large series of 500 patients (including melanoma patients) with spinal metastasis who received single-fraction SRS treatment showed a long-term tumor control of 90%, and long-term pain control of 85% (107). A study focused on melanoma patients also showed axial and radicular pain improved in 27 of 28 patients (96%) treated with radiosurgery (99).

Melanoma has a marked predilection for the liver, particularly, ocular melanoma. Liver metastasis can occur in 15–20% of metastatic cutaneous melanoma (108, 109), and up to 95% of metastatic ocular melanoma (110, 111). With either type of melanoma, liver metastasis is attributed to a grim prognosis and is often the cause of death (112, 113). For those with chemorefractory liver metastases, liver-directed therapy is a preferred approach to reduce tumor burden and prolong overall survival. Unfortunately, only a very small subset (~9%) of patients are eligible for resection (114, 115). Treatment options for unresectable hepatic metastatic melanoma have historically been poor. Recent studies utilizing Yttrium-90 (^{90}Y) radioembolization have led to encouraging results (114, 116–118). This is a special form of radiation that was initially established for the treatment of hepatocellular carcinoma and liver metastasis (119–121). The first study in 2009 by Kennedy et al. on 11 uveal melanoma patients reported a strikingly high response rate of 77% with a 1-year survival of 80% (119). Further experiences suggest that it is an effective and safe option for managing hepatic metastasis from melanoma, with a high response rate (partial response and stable disease) in 80–90% (116–118, 122, 123). Given the hypervascular and aggressive nature of melanoma liver metastases, locoregional treatment with selective internal radiation therapy (SIRT) appears to be a reasonable approach at reducing disease progression. Median overall survival ranges from 7.6 to 10.1 months, substantially improved over the expected >3 month historical benchmark (124). However, large, randomized trials are warranted in order to validate radioembolization for melanoma liver metastasis.

RT with Concomitant Agents

There have been substantial recent advancements in the management of advanced stage melanoma, such as BRAF inhibitor and immunotherapy (125–127). This stimulates the interest of combining such agents with radiation.

BRAF mutations occur in approximately 40–70% of patients, leading to constitutive and uncontrolled cell proliferation, as well as deregulated apoptosis (128, 129). The development of BRAF inhibitors (i.e., vemurafenib, dabrafenib) has led to a significant improvement in the overall survival among patients who harbor

this mutation (125, 130, 131). Interestingly, BRAF inhibitor was found to have radio sensitization effect (132, 133). However, the radiosensitization effect of BRAF inhibitor also increased the risk of skin toxicities with radiation (133–136). Due to the minimum skin dose from SRS, several studies that evaluated BRAF inhibitor with SRS for patients with brain metastases reported favorable outcome (137–139). Studies that directly compared outcomes of patients treated with SRS alone and SRS with BRAF inhibitor suggest that there indeed may be a survival benefit of combination therapy (140–142). However, it seems that because of the radiosensitization effect, increased toxicity other than skin toxicity may also be induced, such as radionecrosis (141). As a result, consensus guidelines from the Eastern Cooperative Group (ECOG) were recently published documenting severe toxicities reported in 27 publications in which patients received a BRAF inhibitor in combination with RT. Based on this review, recommendations for combination therapy include holding BRAF inhibitor for at least 3 days before and after fractionated RT and at least 1 day before and after SRS. There were no fatal reactions documented with RT doses less than 4 Gy per fraction. More prospective trials are necessary to further clarify the optimal timing of BRAF inhibition with RT (143).

In recent years, there is great enthusiasm on the combination of RT with immunotherapy for patients with metastatic melanoma. Recent advances have demonstrated the efficacy of immunotherapy in the treatment of melanoma (126, 127). Several immune therapy strategies have achieved great clinical success in metastatic melanoma, resulting in overall survival improvement (126, 144–149). There are multiple rationales to support the combination of radiation with immunotherapy, and such a combination may lead to a synergistic effect. Radiation is a promising immunological adjuvant and a complex modifier of the tumor microenvironment. Radiation-induced damage in the tumor and normal tissue is affected by various regulatory immune mechanisms (150). Radiation, in particular hypofractionated radiation, can induce the expression of checkpoints, such as PD-L1, PD-L2, and CTLA-4 (151–153). Hence, removing the immune inhibition leads to enhanced tumor control effect. RT promotes tumor cell death, releasing tumor debris and tumor antigens. Radiation treatment has the capacity to prime an adaptive T-cell-mediated immune response, through mechanisms that enhance antigen presentation, activation of dendritic cells, and cross-presentation of tumor-associate antigens (154–156). Besides local effect, radiation may also impact systemic response. Abscopal effect refers to the infrequently reported tumor regression of a secondary site following RT to a separate primary site (157–161). One recent report analyzed 21 patients with advanced melanoma treated with ipilimumab followed by RT and observed an abscopal response in 11 patients (52%) with the median time of 1 month from RT to response. Median overall survival for those patients who had an abscopal response was 22.4 months versus 8.3 months for those without a response. Larger prospective studies are required to bolster this small but impressive report (160). This effect is believed mediate through immune response. Seromic analysis and immunologic correlates of the abscopal effect in a patient with melanoma showed antigenic targets with increased antibody responses following RT (159). Recently, Hiniker et al. reported the result from a prospective trial including 22 patients with Stage IV melanoma treated with palliative RT and four cycles of ipilimumab. The primary objective is assessing safety and efficacy of this combination (162). RT was delivered within 5 days following initiation of immunotherapy. The combination of treatments was well tolerated without

unexpected toxicities. Three patients had complete responses and three had partial responses, suggesting further investigation of the combination of RT with immunotherapy in patients with Stage IV melanoma (162). Similarly, early experiences also showed that dramatic responses have also been shown in the combination of RT with PD-1 or PDL-1 blockade in patients with advanced melanoma (163). Currently, sufficient evidence on the optimal RT dose, schedule, and temporal relationship with immune therapy is lacking. Great efforts are dedicated to address these questions; currently there are multiple open clinical trials evaluating various combinations of RT (EBRT, SRS, SBRT, or radiospheres) with immunotherapy (ipilimumab, nivolumab, atezolizumab, etc.) (www.ClinicalTrials.gov).

Perspectives and Conclusions

RT clearly will continue to play an important role in the management of melanoma. With the advances in the more effective systemic therapy and immune therapy, there is great enthusiasm for combining radiation with systemic therapy. Currently, only a few small studies reported the combination of radiation and immune therapy. Early data suggest that such strategies may improve treatment outcome but also increase adverse effects. There are currently several open clinical trials evaluating various combinations of RT with immunotherapy. The optimal combination, timing, and fractionation schedule of radiation will be further defined with the results of these ongoing trials. However, it is clear that further advances in the treatment of melanoma will be multidisciplinary.

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References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016;66(1):7–30. http://dx.doi.org/10.3322/caac.21332
2. Garbe C, Peris K, Hauschild A, Saiag P, Middleton M, Spatz A, et al. Diagnosis and treatment of melanoma. European consensus-based interdisciplinary guideline—Update 2012. Eur J Cancer. 2012;48(15):2375–90. http://dx.doi.org/10.1016/j.ejca.2012.06.013
3. Espenel S, Vallard A, Rancoule C, Garcia MA, Guy JB, Chargari C, et al. Melanoma: Last call for radiotherapy. Crit Rev Oncol/Hematol. 2017;110:13–19. http://dx.doi.org/10.1016/j.critrevonc.2016.12.003
4. Zabierowski SE, Herlyn M. Melanoma stem cells: The dark seed of melanoma. J Clin Oncol. 2008;26(17):2890–4. http://dx.doi.org/10.1200/JCO.2007.15.5465
5. Jochemsen AG. Reactivation of p53 as therapeutic intervention for malignant melanoma. Curr Opin Oncol. 2014;26(1):114–19. http://dx.doi.org/10.1097/CCO.0000000000000033

6. Dewey DL. The radiosensitivity of melanoma cells in culture. Br J Radiol. 1971;44(526):816–17. http://dx.doi.org/10.1259/0007-1285-44-526-816
7. Barranco SC, Romsdahl MM, Humphrey RM. The radiation response of human malignant melanoma cells grown in vitro. Cancer Res. 1971;31(6):830–3.
8. Sause WT, Cooper JS, Rush S, Ago CT, Cosmatos D, Coughlin CT, et al. Fraction size in external beam radiation therapy in the treatment of melanoma. Int J Radiat Oncol Biol Phys. 1991;20(3):429–32. http://dx.doi.org/10.1016/0360-3016(91)90053-7
9. Dvorak E, Haas RE, Liebner EJ. Contribution of radiotherapy to the management of malignant melanoma. A ten year experience at the University of Illinois Hospital in Chicago. Neoplasma. 1993;40(6):387–99.
10. Fenig E, Eidelevich E, Njuguna E, Katz A, Gutman H, Sulkes A, et al. Role of radiation therapy in the management of cutaneous malignant melanoma. Am J Clin Oncol. 1999;22(2):184–6. http://dx.doi.org/10.1097/00000421-199904000-00017
11. Chang DT, Amdur RJ, Morris CG, Mendenhall WM. Adjuvant radiotherapy for cutaneous melanoma: Comparing hypofractionation to conventional fractionation. Int J Radiat Oncol Biol Phys. 2006;66(4):1051–5. http://dx.doi.org/10.1016/j.ijrobp.2006.05.056
12. Strojan P, Jancar B, Cemazar M, Perme MP, Hocevar M. Melanoma metastases to the neck nodes: Role of adjuvant irradiation. Int J Radiat Oncol Biol Phys. 2010;77(4):1039–45. http://dx.doi.org/10.1016/j.ijrobp.2009.06.071
13. Noda SE, Lautenschlaeger T, Siedow MR, Patel DR, El-Jawahri A, Suzuki Y, et al. Technological advances in radiation oncology for central nervous system tumors. Semin Radiat Oncol. 2009;19(3):179–86. http://dx.doi.org/10.1016/j.semradonc.2009.02.006
14. Barnett GH, Linskey ME, Adler JR, Cozzens JW, Friedman WA, Heilbrun MP, et al. Stereotactic radiosurgery—An organized neurosurgery-sanctioned definition. J Neurosurg. 2007;106(1):1–5. http://dx.doi.org/10.3171/jns.2007.106.1.1
15. Kvaskoff M, Siskind V, Green AC. Risk factors for lentigo maligna melanoma compared with superficial spreading melanoma: A case-control study in Australia. Arch Dermatol. 2012;148(2):164–70. http://dx.doi.org/10.1001/archdermatol.2011.291
16. Weedon D. Melanoma and other melanocytic skin lesions. Curr Top Pathol. 1985;74:1–55. http://dx.doi.org/10.1007/978-3-642-69574-2_1
17. De Groot WP. Provisional results of treatment of the melanose precancereuse circonscrite Dubreuilh by Bucky-rays. Dermatologica. 1968;136(5):429–31. http://dx.doi.org/10.1159/000254133
18. Farshad A, Burg G, Panizzon R, Dummer R. A retrospective study of 150 patients with lentigo maligna and lentigo maligna melanoma and the efficacy of radiotherapy using Grenz or soft X-rays. Br J Dermatol. 2002;146(6):1042–6. http://dx.doi.org/10.1046/j.1365-2133.2002.04750.x
19. Panizzon R. Radiotherapy of lentigo maligna and lentigo maligna melanoma. Skin Cancer. 1999;14. 203–207.
20. Schmid-Wendtner MH, Brunner B, Konz B, Kaudewitz P, Wendtner CM, Peter RU, et al. Fractionated radiotherapy of lentigo maligna and lentigo maligna melanoma in 64 patients. J Am Acad Dermatol. 2000;43(3):477–82. http://dx.doi.org/10.1067/mjd.2000.106241
21. Hedblad MA, Mallbris L. Grenz ray treatment of lentigo maligna and early lentigo maligna melanoma. J Am Acad Dermatol. 2012;67(1):60–8. http://dx.doi.org/10.1016/j.jaad.2011.06.029
22. Fogarty GB, Hong A, Scolyer RA, Lin E, Haydu L, Guitera P, et al. Radiotherapy for lentigo maligna: A literature review and recommendations for treatment. Br J Dermatol. 2014;170(1):52–8. http://dx.doi.org/10.1111/bjd.12611
23. Read T, Noonan C, David M, Wagels M, Foote M, Schaider H, et al. A systematic review of non-surgical treatments for lentigo maligna. J Eur Acad Dermatol Venereol. 2016;30(5):748–53. http://dx.doi.org/10.1111/jdv.13252
24. Spencer KR, Mehnert JM. Mucosal melanoma: Epidemiology, biology and treatment. Cancer Treat Res. 2016;167:295–320. http://dx.doi.org/10.1007/978-3-319-22539-5_13
25. Meleti M, Leemans CR, de Bree R, Vescovi P, Sesenna E, van der Waal I. Head and neck mucosal melanoma: Experience with 42 patients, with emphasis on the role of postoperative radiotherapy. Head Neck. 2008;30(12):1543–51. http://dx.doi.org/10.1002/hed.20901
26. Bachar G, Goldstein DP, Shah M, Tandon A, Ringash J, Pond G, et al. Esthesioneuroblastoma: The Princess Margaret Hospital experience. Head Neck. 2008;30(12):1607–14. http://dx.doi.org/10.1002/hed.20920

27. Krengli M, Masini L, Kaanders JH, Maingon P, Oei SB, Zouhair A, et al. Radiotherapy in the treatment of mucosal melanoma of the upper aerodigestive tract: Analysis of 74 cases. A Rare Cancer Network study. Int J Radiat Oncol Biol Phys. 2006;65(3):751–9. http://dx.doi.org/10.1016/j.ijrobp.2006.01.016
28. Moreno MA, Roberts DB, Kupferman ME, DeMonte F, El-Naggar AK, Williams M, et al. Mucosal melanoma of the nose and paranasal sinuses, a contemporary experience from the M. D. Anderson Cancer Center. Cancer. 2010;116(9):2215–23. http://dx.doi.org/10.1002/cncr.24976
29. Benlyazid A, Thariat J, Temam S, Malard O, Florescu C, Choussy O, et al. Postoperative radiotherapy in head and neck mucosal melanoma: A GETTEC study. Arch Otolaryngol Head Neck Surg. 2010;136(12):1219–25. http://dx.doi.org/10.1001/archoto.2010.217
30. Demizu Y, Fujii O, Terashima K, Mima M, Hashimoto N, Niwa Y, et al. Particle therapy for mucosal melanoma of the head and neck. A single-institution retrospective comparison of proton and carbon ion therapy. Strahlenther Onkol. 2014;190(2):186–91. http://dx.doi.org/10.1007/s00066-013-0489-9
31. Gilligan D, Slevin NJ. Radical radiotherapy for 28 cases of mucosal melanoma in the nasal cavity and sinuses. Br J Radiol. 1991;64(768):1147–50. http://dx.doi.org/10.1259/0007-1285-64-768-1147
32. Wu AJ, Gomez J, Zhung JE, Chan K, Gomez DR, Wolden SL, et al. Radiotherapy after surgical resection for head and neck mucosal melanoma. Am J Clin Oncol. 2010;33(3):281–5.
33. Wada H, Nemoto K, Ogawa Y, Hareyama M, Yoshida H, Takamura A, et al. A multi-institutional retrospective analysis of external radiotherapy for mucosal melanoma of the head and neck in Northern Japan. Int J Radiat Oncol Biol Phys. 2004;59(2):495–500. http://dx.doi.org/10.1016/j.ijrobp.2003.11.013
34. Singh AD, Turell ME, Topham AK. Uveal melanoma: Trends in incidence, treatment, and survival. Ophthalmology. 2011;118(9):1881–5. http://dx.doi.org/10.1016/j.ophtha.2011.01.040
35. Munzenrider JE. Uveal melanomas. Conservation treatment. Hematol Oncol Clin North Am. 2001;15(2):389–402. http://dx.doi.org/10.1016/S0889-8588(05)70219-7
36. Moore RF. Choroidal sarcoma treated by the intraocular insertion of radon seeds. Br J Ophthalmol. 1930;14(4):145–52. http://dx.doi.org/10.1136/bjo.14.4.145
37. Stallard HB. Radiotherapy for malignant melanoma of the choroid. Br J Ophthalmol. 1966;50(3):147–55. http://dx.doi.org/10.1136/bjo.50.3.147
38. American Brachytherapy Society – Ophthalmic Oncology Task Force. Electronic address pec, Committee AO. The American Brachytherapy Society consensus guidelines for plaque brachytherapy of uveal melanoma and retinoblastoma. Brachytherapy. 2014;13(1):1–14. http://dx.doi.org/10.1016/j.brachy.2013.11.008
39. Melia BM, Abramson DH, Albert DM, Boldt HC, Earle JD, Hanson WF, et al. Collaborative ocular melanoma study (COMS) randomized trial of I-125 brachytherapy for medium choroidal melanoma. I. Visual acuity after 3 years COMS report no. 16. Ophthalmology. 2001;108(2):348–66. http://dx.doi.org/10.1016/S0161-6420(00)00526-1
40. Perez BA, Mettu P, Vajzovic L, Rivera D, Alkaissi A, Steffey BA, et al. Uveal melanoma treated with iodine-125 episcleral plaque: An analysis of dose on disease control and visual outcomes. Int J Radiat Oncol Biol Phys. 2014;89(1):127–36. http://dx.doi.org/10.1016/j.ijrobp.2014.01.026
41. Jones R, Gore E, Mieler W, Murray K, Gillin M, Albano K, et al. Posttreatment visual acuity in patients treated with episcleral plaque therapy for choroidal melanomas: Dose and dose rate effects. Int J Radiat Oncol Biol Phys. 2002;52(4):989–95. http://dx.doi.org/10.1016/S0360-3016(01)02723-7
42. Gragoudas E, Li W, Goitein M, Lane AM, Munzenrider JE, Egan KM. Evidence-based estimates of outcome in patients irradiated for intraocular melanoma. Arch Ophthalmol. 2002;120(12):1665–71. http://dx.doi.org/10.1001/archopht.120.12.1665
43. Gragoudas ES, Lane AM, Munzenrider J, Egan KM, Li W. Long-term risk of local failure after proton therapy for choroidal/ciliary body melanoma. Trans Am Ophthalmol Soc. 2002;100:43–8; discussion 8–9.
44. Gragoudas ES, Lane AM, Regan S, Li W, Judge HE, Munzenrider JE, et al. A randomized controlled trial of varying radiation doses in the treatment of choroidal melanoma. Arch Ophthalmol. 2000;118(6):773–8. http://dx.doi.org/10.1001/archopht.118.6.773
45. Wang Z, Nabhan M, Schild SE, Stafford SL, Petersen IA, Foote RL, et al. Charged particle radiation therapy for uveal melanoma: A systematic review and meta-analysis. Int J Radiat Oncol Biol Phys. 2013;86(1):18–26. http://dx.doi.org/10.1016/j.ijrobp.2012.08.026

46. Rickhey M, Moravek Z, Eilles C, Koelbl O, Bogner L. 18F-FET-PET-based dose painting by numbers with protons. Strahlenther Onkol. 2010;186(6):320–6. http://dx.doi.org/10.1007/s00066-010-2014-8
47. Tokuuye K, Akine Y, Sumi M, Kagami Y, Ikeda H, Kaneko A. Fractionated stereotactic radiotherapy for choroidal melanoma. Radiother Oncol. 1997;43(1):87–91. http://dx.doi.org/10.1016/S0167-8140(97)01910-5
48. Dieckmann K, Georg D, Zehetmayer M, Bogner J, Georgopoulos M, Pötter R. LINAC based stereotactic radiotherapy of uveal melanoma: 4 years clinical experience. Radiother Oncol. 2003;67(2):199–206. http://dx.doi.org/10.1016/S0167-8140(02)00345-6
49. Wackernagel W, Holl E, Tarmann L, Mayer C, Avian A, Schneider M, et al. Local tumour control and eye preservation after gamma-knife radiosurgery of choroidal melanomas. Br J Ophthalmol. 2014;98(2):218–23. http://dx.doi.org/10.1136/bjophthalmol-2013-304031
50. Wackernagel W, Holl E, Tarmann L, Avian A, Schneider MR, Kapp K, et al. Visual acuity after Gamma-Knife radiosurgery of choroidal melanomas. Br J Ophthalmol. 2013;97(2):153–8. http://dx.doi.org/10.1136/bjophthalmol-2012-302399
51. Haji Mohd Yasin NA, Gray AR, Bevin TH, Kelly LE, Molteno AC. Choroidal melanoma treated with stereotactic fractionated radiotherapy and prophylactic intravitreal bevacizumab: The Dunedin Hospital experience. J Med Imaging Radiat Oncol. 2016;60(6):756–63. http://dx.doi.org/10.1111/1754-9485.12489
52. Sarici AM, Pazarli H. Gamma-knife-based stereotactic radiosurgery for medium- and large-sized posterior uveal melanoma. Graefes Arch Clin Exp Ophthalmol. 2013;251(1):285–94. http://dx.doi.org/10.1007/s00417-012-2144-z
53. Krema H, Somani S, Sahgal A, Xu W, Heydarian M, Payne D, et al. Stereotactic radiotherapy for treatment of juxtapapillary choroidal melanoma: 3-year follow-up. Br J Ophthalmol. 2009;93(9):1172–6. http://dx.doi.org/10.1136/bjo.2008.153429
54. Ballo MT, Zagars GK, Gershenwald JE, Lee JE, Mansfield PF, Kim KB, et al. A critical assessment of adjuvant radiotherapy for inguinal lymph node metastases from melanoma. Ann Surg Oncol. 2004;11(12):1079–84. http://dx.doi.org/10.1245/ASO.2004.12.039
55. Dickson RJ. Malignant melanoma; a combined surgical and radiotherapeutic approach. Am J Roentgenol Radium Ther Nucl Med. 1958;79(6):1063–70.
56. Harwood AR, Cummings BJ. Radiotherapy for mucosal melanomas. Int J Radiat Oncol Biol Phys. 1982;8(7):1121–6. http://dx.doi.org/10.1016/0360-3016(82)90058-X
57. Stevens G, Thompson JF, Firth I, O'Brien CJ, McCarthy WH, Quinn MJ. Locally advanced melanoma: Results of postoperative hypofractionated radiation therapy. Cancer. 2000;88(1):88–94. http://dx.doi.org/10.1002/(SICI)1097-0142(20000101)88:1%3C88::AID-CNCR13%3E3.0.CO;2-K
58. Strom T, Caudell JJ, Han D, Zager JS, Yu D, Cruse CW, et al. Radiotherapy influences local control in patients with desmoplastic melanoma. Cancer. 2014;120(9):1369–78. http://dx.doi.org/10.1002/cncr.28412
59. Mendenhall WM, Shaw C, Amdur RJ, Kirwan J, Morris CG, Werning JW. Surgery and adjuvant radiotherapy for cutaneous melanoma considered high-risk for local-regional recurrence. Am J Otolaryngol. 2013;34(4):320–2. http://dx.doi.org/10.1016/j.amjoto.2012.12.014
60. Agrawal S, Kane JM, 3rd, Guadagnolo BA, Kraybill WG, Ballo MT. The benefits of adjuvant radiation therapy after therapeutic lymphadenectomy for clinically advanced, high-risk, lymph node-metastatic melanoma. Cancer. 2009;115(24):5836–44. http://dx.doi.org/10.1002/cncr.24627
61. Ballo MT, Bonnen MD, Garden AS, Myers JN, Gershenwald JE, Zagars GK, et al. Adjuvant irradiation for cervical lymph node metastases from melanoma. Cancer. 2003;97(7):1789–96. http://dx.doi.org/10.1002/cncr.11243
62. Ballo MT, Strom EA, Zagars GK, Bedikian AY, Prieto VG, Mansfield PF, et al. Adjuvant irradiation for axillary metastases from malignant melanoma. Int J Radiat Oncol Biol Phys. 2002;52(4):964–72. http://dx.doi.org/10.1016/S0360-3016(01)02742-0
63. Strojan P. Role of radiotherapy in melanoma management. Radiol Oncol. 2010;44(1):1–12. http://dx.doi.org/10.2478/v10019-010-0008-x
64. Henderson MA, Burmeister BH, Ainslie J, Fisher R, Di Iulio J, Smithers BM, et al. Adjuvant lymph-node field radiotherapy versus observation only in patients with melanoma at high risk of further

lymph-node field relapse after lymphadenectomy (ANZMTG 01.02/TROG 02.01): 6-year follow-up of a phase 3, randomised controlled trial. Lancet Oncol. 2015;16(9):1049–60. http://dx.doi.org/10.1016/S1470-2045(15)00187-4
65. Fogarty GB, Hong A. Radiation therapy for advanced and metastatic melanoma. J Surg Oncol. 2014;109(4):370–5. http://dx.doi.org/10.1002/jso.23509
66. Hellman S, Weichselbaum RR. Oligometastases. J Clin Oncol. 1995;13(1):8–10. http://dx.doi.org/10.1200/JCO.1995.13.1.8
67. Sosman JA, Moon J, Tuthill RJ, Warneke JA, Vetto JT, Redman BG, et al. A phase 2 trial of complete resection for stage IV melanoma: Results of Southwest Oncology Group Clinical Trial S9430. Cancer. 2011;117(20):4740–6. http://dx.doi.org/10.1002/cncr.26111
68. Ollila DW, Essner R, Wanek LA, Morton DL. Surgical resection for melanoma metastatic to the gastrointestinal tract. Arch Surg. 1996;131(9):975–9; 9–80.
69. Agrawal S, Yao TJ, Coit DG. Surgery for melanoma metastatic to the gastrointestinal tract. Ann Surg Oncol. 1999;6(4):336–44. http://dx.doi.org/10.1007/s10434-999-0336-5
70. Harpole DH, Jr., Johnson CM, Wolfe WG, George SL, Seigler HF. Analysis of 945 cases of pulmonary metastatic melanoma. J Thorac Cardiovasc Surg. 1992;103(4):743–8; discussion 8–50.
71. Milano MT, Katz AW, Muhs AG, Philip A, Buchholz DJ, Schell MC, et al. A prospective pilot study of curative-intent stereotactic body radiation therapy in patients with 5 or fewer oligometastatic lesions. Cancer. 2008;112(3):650–8. http://dx.doi.org/10.1002/cncr.23209
72. Salama JK, Hasselle MD, Chmura SJ, Malik R, Mehta N, Yenice KM, et al. Stereotactic body radiotherapy for multisite extracranial oligometastases: Final report of a dose escalation trial in patients with 1 to 5 sites of metastatic disease. Cancer. 2012;118(11):2962–70. http://dx.doi.org/10.1002/cncr.26611
73. Bafaloukos D, Gogas H. The treatment of brain metastases in melanoma patients. Cancer Treat Rev. 2004;30(6):515–20. http://dx.doi.org/10.1016/j.ctrv.2004.05.001
74. Sampson JH, Carter JH, Jr., Friedman AH, Seigler HF. Demographics, prognosis, and therapy in 702 patients with brain metastases from malignant melanoma. J Neurosurg. 1998;88(1):11–20. http://dx.doi.org/10.3171/jns.1998.88.1.0011
75. Barth A, Wanek LA, Morton DL. Prognostic factors in 1,521 melanoma patients with distant metastases. J Am Coll Surg. 1995;181(3):193–201.
76. Andrews DW. Current neurosurgical management of brain metastases. Semin Oncol. 2008;35(2):100–7. http://dx.doi.org/10.1053/j.seminoncol.2007.12.003
77. Thomas SS, Dunbar EM. Modern multidisciplinary management of brain metastases. Curr Oncol Rep. 2010;12(1):34–40. http://dx.doi.org/10.1007/s11912-009-0073-8
78. Chu FC, Hilaris BB. Value of radiation theray in the management of intracranial metastases. Cancer. 1961;14:577–81. http://dx.doi.org/10.1002/1097-0142(199005/06)14:3%3C577::AID-CNCR2820140318%3E3.0.CO;2-F
79. Meyners T, Heisterkamp C, Kueter JD, Veninga T, Stalpers LJ, Schild SE, et al. Prognostic factors for outcomes after whole-brain irradiation of brain metastases from relatively radioresistant tumors: A retrospective analysis. BMC Cancer. 2010;10:582. http://dx.doi.org/10.1186/1471-2407-10-582
80. de la Fuente M, Beal K, Carvajal R, Kaley TJ. Whole-brain radiotherapy in patients with brain metastases from melanoma. CNS Oncol. 2014;3(6):401–6. http://dx.doi.org/10.2217/cns.14.40
81. Chang EL, Wefel JS, Hess KR, Allen PK, Lang FF, Kornguth DG, et al. Neurocognition in patients with brain metastases treated with radiosurgery or radiosurgery plus whole-brain irradiation: A randomised controlled trial. Lancet Oncol. 2009;10(11):1037–44. http://dx.doi.org/10.1016/S1470-2045(09)70263-3
82. Brown PD, Jaeckle K, Ballman KV, Farace E, Cerhan JH, Anderson SK, et al. Effect of radiosurgery alone vs radiosurgery with whole brain radiation therapy on cognitive function in patients with 1 to 3 brain metastases: A randomized clinical trial. JAMA. 2016;316(4):401–9. http://dx.doi.org/10.1001/jama.2016.9839
83. DiLuna ML, King JT, Jr., Knisely JP, Chiang VL. Prognostic factors for survival after stereotactic radiosurgery vary with the number of cerebral metastases. Cancer. 2007;109(1):135–45. http://dx.doi.org/10.1002/cncr.22367

84. Manon R, O'Neill A, Knisely J, Werner-Wasik M, Lazarus HM, Wagner H, et al. Phase II trial of radiosurgery for one to three newly diagnosed brain metastases from renal cell carcinoma, melanoma, and sarcoma: An Eastern Cooperative Oncology Group study (E 6397). J Clin Oncol. 2005;23(34):8870–6. http://dx.doi.org/10.1200/JCO.2005.01.8747
85. Mathieu D, Kondziolka D, Cooper PB, Flickinger JC, Niranjan A, Agarwala S, et al. Gamma knife radiosurgery for malignant melanoma brain metastases. Clin Neurosurg. 2007;54:241–7. http://dx.doi.org/10.1227/01.NEU.0000255342.10780.52
86. Clarke JW, Register S, McGregor JM, Grecula JC, Mayr NA, Wang JZ, et al. Stereotactic radiosurgery with or without whole brain radiotherapy for patients with a single radioresistant brain metastasis. Am J Clin Oncol. 2010;33(1):70–4. http://dx.doi.org/10.1097/COC.0b013e31819ccc8c
87. Powell JW, Chung CT, Shah HR, Canute GW, Hodge CJ, Bassano DA, et al. Gamma Knife surgery in the management of radioresistant brain metastases in high-risk patients with melanoma, renal cell carcinoma, and sarcoma. J Neurosurg. 2008;109(Suppl):122–8.
88. Lo SS, Clarke JW, Grecula JC, McGregor JM, Mayr NA, Cavaliere R, et al. Stereotactic radiosurgery alone for patients with 1–4 radioresistant brain metastases. Med Oncol. 2011;28(Suppl 1):S439–44. http://dx.doi.org/10.1007/s12032-010-9670-5
89. Choong ES, Lo S, Drummond M, Fogarty GB, Menzies AM, Guminski A, et al. Survival of patients with melanoma brain metastasis treated with stereotactic radiosurgery and active systemic drug therapies. Eur J Cancer. 2017;75:169–78. http://dx.doi.org/10.1016/j.ejca.2017.01.007
90. Feng R, Oermann EK, Shrivastava R, Gold A, Collins BT, Kondziolka D, et al. Stereotactic radiosurgery (SRS) for melanoma brain metastases: A comprehensive clinical case series. World Neurosurg. 2017;100:297–304. http://dx.doi.org/10.1016/j.wneu.2017.01.014
91. Yamamoto M, Serizawa T, Shuto T, Akabane A, Higuchi Y, Kawagishi J, et al. Stereotactic radiosurgery for patients with multiple brain metastases (JLGK0901): A multi-institutional prospective observational study. Lancet Oncol. 2014;15(4):387–95. http://dx.doi.org/10.1016/S1470-2045(14)70061-0
92. Rava P, Leonard K, Sioshansi S, Curran B, Wazer DE, Cosgrove GR, et al. Survival among patients with 10 or more brain metastases treated with stereotactic radiosurgery. J Neurosurg. 2013;119(2):457–62. http://dx.doi.org/10.3171/2013.4.JNS121751
93. Nichol A, Ma R, Hsu F, Gondara L, Carolan H, Olson R, et al. Volumetric radiosurgery for 1 to 10 brain metastases: A multicenter, single-arm, phase 2 study. Int J Radiat Oncol Biol Phys. 2016;94(2):312–21. http://dx.doi.org/10.1016/j.ijrobp.2015.10.017
94. 8 Gy single fraction radiotherapy for the treatment of metastatic skeletal pain: Randomised comparison with a multifraction schedule over 12 months of patient follow-up. Bone Pain Trial Working Party. Radiother Oncol. 1999;52(2):111–21. http://dx.doi.org/10.1016/S0167-8140(99)00097-3
95. Rades D, Stalpers LJ, Hulshof MC, Zschenker O, Alberti W, Koning CC. Effectiveness and toxicity of single-fraction radiotherapy with 1 x 8 Gy for metastatic spinal cord compression. Radiother Oncol. 2005;75(1):70–3. http://dx.doi.org/10.1016/j.radonc.2004.12.013
96. Maranzano E, Bellavita R, Rossi R, De Angelis V, Frattegiani A, Bagnoli R, et al. Short-course versus split-course radiotherapy in metastatic spinal cord compression: Results of a phase III, randomized, multicenter trial. J Clin Oncol. 2005;23(15):3358–65. http://dx.doi.org/10.1200/JCO.2005.08.193
97. Chow E, Harris K, Fan G, Tsao M, Sze WM. Palliative radiotherapy trials for bone metastases: A systematic review. J Clin Oncol. 2007;25(11):1423–36. http://dx.doi.org/10.1200/JCO.2006.09.5281
98. Lutz S, Berk L, Chang E, Chow E, Hahn C, Hoskin P, et al. Palliative radiotherapy for bone metastases: An ASTRO evidence-based guideline. Int J Radiat Oncol Biol Phys. 2011;79(4):965–76. http://dx.doi.org/10.1016/j.ijrobp.2010.11.026
99. Gerszten PC, Burton SA, Quinn AE, Agarwala SS, Kirkwood JM. Radiosurgery for the treatment of spinal melanoma metastases. Stereotact Funct Neurosurg. 2005;83(5–6):213–21. http://dx.doi.org/10.1159/000091952
100. Janjan N, Lutz ST, Bedwinek JM, Hartsell WF, Ng A, Pieters RS, et al. Therapeutic guidelines for the treatment of bone metastasis: A report from the American College of Radiology Appropriateness Criteria Expert Panel on Radiation Oncology. J Palliat Med. 2009;12(5):417–26. http://dx.doi.org/10.1089/jpm.2009.9633
101. Rule W, Timmerman R, Tong L, Abdulrahman R, Meyer J, Boike T, et al. Phase I dose-escalation study of stereotactic body radiotherapy in patients with hepatic metastases. Ann Surg Oncol. 2011;18(4):1081–7. http://dx.doi.org/10.1245/s10434-010-1405-5

102. Lo SS, Teh BS, Mayr NA, Olencki TE, Wang JZ, Grecula JC, et al. Stereotactic body radiation therapy for oligometastases. Discov Med. 2010;10(52):247–54.
103. Timmerman R, Paulus R, Galvin J, Michalski J, Straube W, Bradley J, et al. Stereotactic body radiation therapy for inoperable early stage lung cancer. JAMA. 2010;303(11):1070–6. http://dx.doi.org/10.1001/jama.2010.261
104. Rusthoven KE, Kavanagh BD, Cardenes H, Stieber VW, Burri SH, Feigenberg SJ, et al. Multi-institutional phase I/II trial of stereotactic body radiation therapy for liver metastases. J Clin Oncol. 2009;27(10):1572–8. http://dx.doi.org/10.1200/JCO.2008.19.6329
105. Hoyer M, Roed H, Traberg Hansen A, Ohlhuis L, Petersen J, Nellemann H, et al. Phase II study on stereotactic body radiotherapy of colorectal metastases. Acta Oncol. 2006;45(7):823–30. http://dx.doi.org/10.1080/02841860600904854
106. Chawla S, Chen Y, Katz AW, Muhs AG, Philip A, Okunieff P, et al. Stereotactic body radiotherapy for treatment of adrenal metastases. Int J Radiat Oncol Biol Phys. 2009;75(1):71–5. http://dx.doi.org/10.1016/j.ijrobp.2008.10.079
107. Gerszten PC, Burton SA, Ozhasoglu C, Welch WC. Radiosurgery for spinal metastases: Clinical experience in 500 cases from a single institution. Spine (Phila Pa 1976). 2007;32(2):193–9. http://dx.doi.org/10.1097/01.brs.0000251863.76595.a2
108. Leiter U, Meier F, Schittek B, Garbe C. The natural course of cutaneous melanoma. J Surg Oncol. 2004;86(4):172–8. http://dx.doi.org/10.1002/jso.20079
109. Cohn-Cedermark G, Mansson-Brahme E, Rutqvist LE, Larsson O, Singnomklao T, Ringborg U. Metastatic patterns, clinical outcome, and malignant phenotype in malignant cutaneous melanoma. Acta Oncol. 1999;38(5):549–57. http://dx.doi.org/10.1080/028418699431122
110. Becker JC, Terheyden P, Kampgen E, Wagner S, Neumann C, Schadendorf D, et al. Treatment of disseminated ocular melanoma with sequential fotemustine, interferon alpha, and interleukin 2. Br J Cancer. 2002;87(8):840–5. http://dx.doi.org/10.1038/sj.bjc.6600521
111. Trout AT, Rabinowitz RS, Platt JF, Elsayes KM. Melanoma metastases in the abdomen and pelvis: Frequency and patterns of spread. World J Radiol. 2013;5(2):25–32. http://dx.doi.org/10.4329/wjr.v5.i2.25
112. Bakalian S, Marshall JC, Logan P, Faingold D, Maloney S, Di Cesare S, et al. Molecular pathways mediating liver metastasis in patients with uveal melanoma. Clin Cancer Res. 2008;14(4):951–6. http://dx.doi.org/10.1158/1078-0432.CCR-06-2630
113. Feldman ED, Pingpank JF, Alexander HR. Regional treatment options for patients with ocular melanoma metastatic to the liver. Ann Surg Oncol. 2004;11(3):290–7. http://dx.doi.org/10.1245/ASO.2004.07.004
114. Gonsalves CF, Eschelman DJ, Sullivan KL, Anne PR, Doyle L, Sato T. Radioembolization as salvage therapy for hepatic metastasis of uveal melanoma: A single-institution experience. AJR Am J Roentgenol. 2011;196(2):468–73. http://dx.doi.org/10.2214/AJR.10.4881
115. Mariani P, Almubarak MM, Kollen M, Wagner M, Plancher C, Audollent R, et al. Radiofrequency ablation and surgical resection of liver metastases from uveal melanoma. Eur J Surg Oncol. 2016;42(5):706–12. http://dx.doi.org/10.1016/j.ejso.2016.02.019
116. Eldredge-Hindy H, Ohri N, Anne PR, Eschelman D, Gonsalves C, Intenzo C, et al. Yttrium-90 microsphere brachytherapy for liver metastases from uveal melanoma: Clinical outcomes and the predictive value of fluorodeoxyglucose positron emission tomography. Am J Clin Oncol. 2016;39(2):189–95. http://dx.doi.org/10.1097/COC.0000000000000033
117. Xing M, Prajapati HJ, Dhanasekaran R, Lawson DH, Kokabi N, Eaton BR, et al. Selective Internal Yttrium-90 Radioembolization Therapy (90Y-SIRT) versus best supportive care in patients with unresectable metastatic melanoma to the liver refractory to systemic therapy: Safety and efficacy cohort study. Am J Clin Oncol. 2017;40(1):27–34. http://dx.doi.org/10.1097/COC.0000000000000109
118. Memon K, Kuzel TM, Vouche M, Atassi R, Lewandowski RJ, Salem R. Hepatic yttrium-90 radioembolization for metastatic melanoma: A single-center experience. Melanoma Res. 2014;24(3):244–51. http://dx.doi.org/10.1097/CMR.0000000000000051
119. Kennedy A. Radioembolization of hepatic tumors. J Gastrointest Oncol. 2014;5(3):178–89.
120. Memon K, Lewandowski RJ, Mulcahy MF, Riaz A, Ryu RK, Sato KT, et al. Radioembolization for neuroendocrine liver metastases: Safety, imaging, and long-term outcomes. Int J Radiat Oncol Biol Phys. 2012;83(3):887–94. http://dx.doi.org/10.1016/j.ijrobp.2011.07.041

121. Dezarn WA, Cessna JT, DeWerd LA, Feng W, Gates VL, Halama J, et al. Recommendations of the American Association of Physicists in Medicine on dosimetry, imaging, and quality assurance procedures for 90Y microsphere brachytherapy in the treatment of hepatic malignancies. Med Phys. 2011;38(8):4824–45. http://dx.doi.org/10.1118/1.3608909
122. Piduru SM, Schuster DM, Barron BJ, Dhanasekaran R, Lawson DH, Kim HS. Prognostic value of 18f-fluorodeoxyglucose positron emission tomography-computed tomography in predicting survival in patients with unresectable metastatic melanoma to the liver undergoing yttrium-90 radioembolization. J Vasc Interv Radiol. 2012;23(7):943–8. http://dx.doi.org/10.1016/j.jvir.2012.04.010
123. Klingenstein A, Haug AR, Zech CJ, Schaller UC. Radioembolization as locoregional therapy of hepatic metastases in uveal melanoma patients. Cardiovasc Intervent Radiol. 2013;36(1):158–65. http://dx.doi.org/10.1007/s00270-012-0373-5
124. Balch CM, Soong SJ, Murad TM, Smith JW, Maddox WA, Durant JR. A multifactorial analysis of melanoma. IV. Prognostic factors in 200 melanoma patients with distant metastases (stage III). J Clin Oncol. 1983;1(2):126–34. http://dx.doi.org/10.1200/JCO.1983.1.2.126
125. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med. 2011;364(26):2507–16. http://dx.doi.org/10.1056/NEJMoa1103782
126. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711–23. http://dx.doi.org/10.1056/NEJMoa1003466
127. Ribas A, Puzanov I, Dummer R, Schadendorf D, Hamid O, Robert C, et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): A randomised, controlled, phase 2 trial. Lancet Oncol. 2015;16(8):908–18. http://dx.doi.org/10.1016/S1470-2045(15)00083-2
128. Dhomen N, Marais R. BRAF signaling and targeted therapies in melanoma. Hematol Oncol Clin North Am. 2009;23(3):529–45, ix. http://dx.doi.org/10.1016/j.hoc.2009.04.001
129. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF gene in human cancer. Nature. 2002;417(6892):949–54. http://dx.doi.org/10.1038/nature00766
130. Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. N Engl J Med. 2010;363(9):809–19. http://dx.doi.org/10.1056/NEJMoa1002011
131. Hauschild A, Grob JJ, Demidov LV, Jouary T, Gutzmer R, Millward M, et al. Dabrafenib in BRAF-mutated metastatic melanoma: A multicentre, open-label, phase 3 randomised controlled trial. Lancet. 2012;380(9839):358–65. http://dx.doi.org/10.1016/S0140-6736(12)60868-X
132. Sambade MJ, Peters EC, Thomas NE, Kaufmann WK, Kimple RJ, Shields JM. Melanoma cells show a heterogeneous range of sensitivity to ionizing radiation and are radiosensitized by inhibition of B-RAF with PLX-4032. Radiother Oncol. 2011;98(3):394–9. http://dx.doi.org/10.1016/j.radonc.2010.12.017
133. Hecht M, Zimmer L, Loquai C, Weishaupt C, Gutzmer R, Schuster B, et al. Radiosensitization by BRAF inhibitor therapy-mechanism and frequency of toxicity in melanoma patients. Ann Oncol. 2015;26(6):1238–44. http://dx.doi.org/10.1093/annonc/mdv139
134. Huang V, Hepper D, Anadkat M, Cornelius L. Cutaneous toxic effects associated with vemurafenib and inhibition of the BRAF pathway. Arch Dermatol. 2012;148(5):628–33. http://dx.doi.org/10.1001/archdermatol.2012.125
135. Merten R, Hecht M, Haderlein M, Distel L, Fietkau R, Heinzerling L, et al. Increased skin and mucosal toxicity in the combination of vemurafenib with radiation therapy. Strahlenther Onkol. 2014;190(12):1169–72. http://dx.doi.org/10.1007/s00066-014-0698-x
136. Peuvrel L, Ruellan AL, Thillays F, Quereux G, Brocard A, Saint-Jean M, et al. Severe radiotherapy-induced extracutaneous toxicity under vemurafenib. Eur J Dermatol. 2013;23(6):879–81.
137. Narayana A, Mathew M, Tam M, Kannan R, Madden KM, Golfinos JG, et al. Vemurafenib and radiation therapy in melanoma brain metastases. J Neurooncol. 2013;113(3):411–16. http://dx.doi.org/10.1007/s11060-013-1127-1

138. Gaudy-Marqueste C, Carron R, Delsanti C, Loundou A, Monestier S, Archier E, et al. On demand Gamma-Knife strategy can be safely combined with BRAF inhibitors for the treatment of melanoma brain metastases. Ann Oncol. 2014;25(10):2086–91. http://dx.doi.org/10.1093/annonc/mdu266
139. Ahmed KA, Freilich JM, Sloot S, Figura N, Gibney GT, Weber JS, et al. LINAC-based stereotactic radiosurgery to the brain with concurrent vemurafenib for melanoma metastases. J Neurooncol. 2015;122(1):121–6. http://dx.doi.org/10.1007/s11060-014-1685-x
140. Ly D, Bagshaw HP, Anker CJ, Tward JD, Grossmann KF, Jensen RL, et al. Local control after stereotactic radiosurgery for brain metastases in patients with melanoma with and without BRAF mutation and treatment. J Neurosurg. 2015;123(2):395–401. http://dx.doi.org/10.3171/2014.9.JNS141425
141. Patel KR, Chowdhary M, Switchenko JM, Kudchadkar R, Lawson DH, Cassidy RJ, et al. BRAF inhibitor and stereotactic radiosurgery is associated with an increased risk of radiation necrosis. Melanoma Res. 2016;26(4):387–94. http://dx.doi.org/10.1097/CMR.0000000000000268
142. Xu Z, Lee CC, Ramesh A, Mueller AC, Schlesinger D, Cohen-Inbar O, et al. BRAF V600E mutation and BRAF kinase inhibitors in conjunction with stereotactic radiosurgery for intracranial melanoma metastases. J Neurosurg. 2017;126(3):726–34. http://dx.doi.org/10.3171/2016.2.JNS1633
143. Anker CJ, Grossmann KF, Atkins MB, Suneja G, Tarhini AA, Kirkwood JM. Avoiding severe toxicity from combined BRAF inhibitor and radiation treatment: Consensus guidelines from the Eastern Cooperative Oncology Group (ECOG). Int J Radiat Oncol Biol Phys. 2016;95(2):632–46. http://dx.doi.org/10.1016/j.ijrobp.2016.01.038
144. Topalian SL, Sznol M, McDermott DF, Kluger HM, Carvajal RD, Sharfman WH, et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. J Clin Oncol. 2014;32(10):1020–30. http://dx.doi.org/10.1200/JCO.2013.53.0105
145. D'Angelo SP, Larkin J, Sosman JA, Lebbé C, Brady B, Neyns B, et al. Efficacy and safety of nivolumab alone or in combination with ipilimumab in patients with mucosal melanoma: A pooled analysis. J Clin Oncol. 2017;35(2):226–35. http://dx.doi.org/10.1200/JCO.2016.67.9258
146. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med. 2015;373(1):23–34. http://dx.doi.org/10.1056/NEJMoa1504030
147. Weber JS, D'Angelo SP, Minor D, Hodi FS, Gutzmer R, Neyns B, et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): A randomised, controlled, open-label, phase 3 trial. Lancet Oncol. 2015;16(4):375–84. http://dx.doi.org/10.1016/S1470-2045(15)70076-8
148. Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med. 2015;372(4):320–30. http://dx.doi.org/10.1056/NEJMoa1412082
149. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 2013;369(2):122–33. http://dx.doi.org/10.1056/NEJMoa1302369
150. Schaue D, McBride WH. Links between innate immunity and normal tissue radiobiology. Radiat Res. 2010;173(4):406–17. http://dx.doi.org/10.1667/RR1931.1
151. Derer A, Spiljar M, Baumler M, Hecht M, Fietkau R, Frey B, et al. Chemoradiation Increases PD-L1 expression in certain melanoma and glioblastoma cells. Front Immunol. 2016;7:610. http://dx.doi.org/10.3389/fimmu.2016.00610
152. Sunshine JC, Nguyen P, Kaunitz G, Cottrell T, Berry S, Esandrio J, et al. PD-L1 Expression in melanoma: A quantitative immunohistochemical antibody comparison. Clin Cancer Res. 2017;23(16):4938–44. http://dx.doi.org/10.1158/1078-0432.CCR-16-1821
153. Buchbinder E, Hodi FS. Cytotoxic T lymphocyte antigen-4 and immune checkpoint blockade. J Clin Invest. 2015;125(9):3377–83. http://dx.doi.org/10.1172/JCI80012
154. Garnett CT, Palena C, Chakraborty M, Tsang KY, Schlom J, Hodge JW. Sublethal irradiation of human tumor cells modulates phenotype resulting in enhanced killing by cytotoxic T lymphocytes. Cancer Res. 2004;64(21):7985–94. http://dx.doi.org/10.1158/0008-5472.CAN-04-1525
155. Goforth R, Salem AK, Zhu X, Miles S, Zhang XQ, Lee JH, et al. Immune stimulatory antigen loaded particles combined with depletion of regulatory T-cells induce potent tumor specific immunity in

a mouse model of melanoma. Cancer Immunol Immunother. 2009;58(4):517–30. http://dx.doi.org/10.1007/s00262-008-0574-6
156. Chajon E, Castelli J, Marsiglia H, De Crevoisier R. The synergistic effect of radiotherapy and immunotherapy: A promising but not simple partnership. Crit Rev Oncol/Hematol. 2017;111:124–32. http://dx.doi.org/10.1016/j.critrevonc.2017.01.017
157. Mole RH. Whole body irradiation; radiobiology or medicine? Br J Radiol. 1953;26(305):234–41. http://dx.doi.org/10.1259/0007-1285-26-305-234
158. Demaria S, Ng B, Devitt ML, Babb JS, Kawashima N, Liebes L, et al. Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. Int J Radiat Oncol Biol Phys. 2004;58(3):862–70. http://dx.doi.org/10.1016/j.ijrobp.2003.09.012
159. Postow MA, Callahan MK, Barker CA, Yamada Y, Yuan J, Kitano S, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. N Engl J Med. 2012;366(10):925–31. http://dx.doi.org/10.1056/NEJMoa1112824
160. Grimaldi AM, Simeone E, Giannarelli D, Muto P, Falivene S, Borzillo V, et al. Abscopal effects of radiotherapy on advanced melanoma patients who progressed after ipilimumab immunotherapy. Oncoimmunology. 2014;3:e28780. http://dx.doi.org/10.4161/onci.28780
161. Stamell EF, Wolchok JD, Gnjatic S, Lee NY, Brownell I. The abscopal effect associated with a systemic anti-melanoma immune response. Int J Radiat Oncol Biol Phys. 2013;85(2):293–5. http://dx.doi.org/10.1016/j.ijrobp.2012.03.017
162. Hiniker SM, Chen DS, Reddy S, Chang DT, Jones JC, Mollick JA, et al. A systemic complete response of metastatic melanoma to local radiation and immunotherapy. Transl Oncol. 2012;5(6):404–7. http://dx.doi.org/10.1593/tlo.12280
163. Mohiuddin M, Park H, Hallmeyer S, Richards J. High-dose radiation as a dramatic, immunological primer in locally advanced melanoma. Cureus. 2015;7(12):e417. http://dx.doi.org/10.7759/cureus.417

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Immune Checkpoint Inhibitors in the Treatment of Melanoma: From Basic Science to Clinical Application

MATTHEW P. RAUSCH[1] • KAREN TARASZKA HASTINGS[2]

[1]Surface Oncology, 50 Hampshire Street, 8th Floor, Cambridge, MA 02139, USA; [2]Department of Basic Medical Sciences, The University of Arizona, College of Medicine–Phoenix, AZ, USA.

Author for correspondence: Karen Taraszka Hastings, The University of Arizona, College of Medicine–Phoenix, 425 N. 5th St., Phoenix, AZ 85004, USA. E-mail: khasting@email.arizona.edu

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Abstract: Immune checkpoint blockade has revolutionized the treatment of patients with advanced melanoma and many other cancers. Blockade of inhibitory receptors, CTLA-4 and PD-1, enhances T-cell-mediated antitumor immune responses, leading to improved survival and durable responses in patients. Based on their mechanism of action, immune checkpoint inhibitors can also induce immune-related adverse events that require careful monitoring and prompt treatment. Despite these successes, only a fraction of patients benefit from immune checkpoint blockade. Basic science approaches and clinical experience are defining predictive biomarkers to identify patients most likely to respond to therapy as well as mechanisms of resistance that limit responses in certain tumors or shorten the duration of response. New approaches and combination therapies are under development to broaden the clinical impact of immune checkpoint blockade by overcoming resistance to therapy and limiting adverse events.

Key words: CTLA-4; Ipilimumab; Nivolumab; PD-1; Pembrolizumab

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Introduction

The development of immune checkpoint inhibitors has transformed the treatment of melanoma (1). Immune checkpoint inhibitors were the first class of therapy shown to improve the overall survival for patients with advanced melanoma. In fact, long-term, durable tumor regression has become a reality for some patients. However, only a subset of melanoma patients respond to immune checkpoint inhibitors, highlighting the need to identify biomarkers that are predictive of response and to develop strategies that overcome resistance. T-cell activation is a complex process that begins with the binding of a specific T-cell receptor (TCR) to its cognate peptide-MHC complex presented on the surface of an antigen-presenting cell (APC). Full T-cell activation requires co-stimulatory signals. CD28 is the major co-stimulatory receptor on T-cells, and by interacting with B7 family ligands CD80 and CD86 on APCs, CD28 promotes enhanced proliferation, IL-2 production, and T-cell survival (2) (Figure 1). In addition, T-cell activation involves the carefully balanced integration of a number of co-inhibitory signals delivered by immune checkpoint receptors. Immune checkpoints are a critical control mechanism to turn off T-cell responses and prevent destructive inflammation. The most extensively studied immune checkpoint receptors are cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) and programmed cell death protein-1 (PD-1).

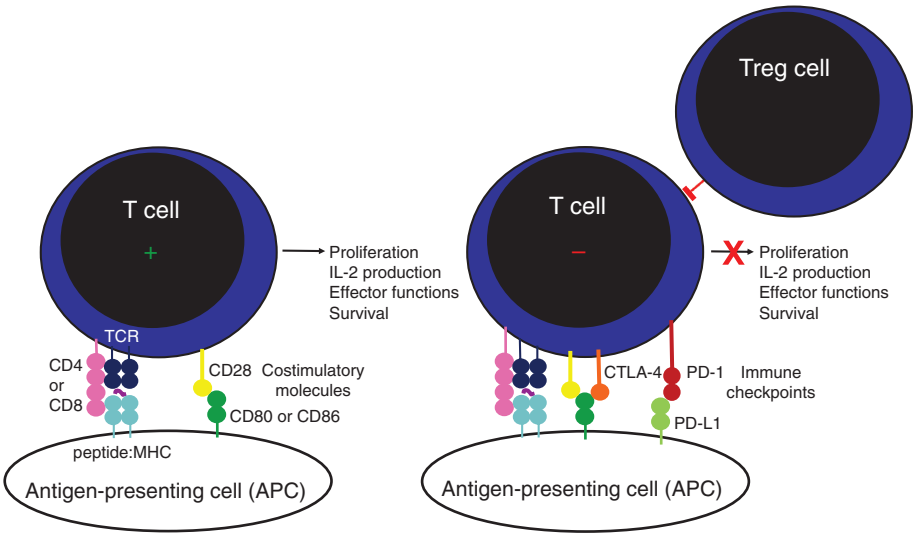

Figure 1 Activation and control of T-cell responses. Interaction of the T-cell receptor (TCR, dark blue) and accessory molecule (CD4 or CD8, pink) on the T-cell with peptide-MHC (purple:light blue) on the APC together with co-stimulatory molecule CD28 (yellow) on the T-cell with CD80 or CD86 (dark green) on the APC results in T-cell activation. Immune checkpoints CTLA-4 (orange) and PD-1 (red) are expressed on T-cells after activation and serve to dampen T-cell responses. Treg cells also suppress T-cell functions. CTLA-4 and PD-1 are targets of immunotherapeutics in melanoma. Blockade of these immune checkpoints augments antitumor T-cell responses.

Biology of CTLA-4

Initially cloned in the 1980s, CTLA-4 is a member of the immunoglobulin (Ig) gene superfamily with homology to CD28 (3). CTLA-4 is expressed on the surface of activated T-cells and regulatory T (Treg) cells. CTLA-4 inhibits T-cell activation during the priming phase of immunity (4–6) (Figure 1). Like CD28, CTLA-4 binds to the B7 ligands CD80 and CD86 on APCs, but unlike its homologue, CTLA-4 binds these ligands with a much higher affinity and does not deliver a positive signal (4, 6–8). Thus, CTLA-4 competitively inhibits the CD28:B7 interaction, leading to attenuation of co-stimulatory signaling. In addition, CTLA-4-expressing cells have been shown to capture and degrade CD80 and CD86 from the APC surface (9). The mechanism of action of CTLA-4-mediated T-cell suppression involves the inhibition of IL-2 production and blockade of cell cycle progression in T-cells following initial activation (5).

The expression of CTLA-4 is tightly regulated and dependent on T-cell activation. Unlike CD28, which is constitutively expressed by all T-cells, CTLA-4 expression is absent from naïve T-cells (10). CTLA-4 is only expressed after T-cell activation with transcript levels becoming detectable 1 h after TCR stimulation (10) and cell surface expression at the immunological synapse showing up 24–48 h post stimulation (6). Furthermore, the strength of T-cell stimulation is directly proportional to the level of CTLA-4 expression (11). In this way, CTLA-4 functions as a T-cell intrinsic inhibitory feedback mechanism that plays a vital role in shutting down T-cell-mediated immune responses. The critical importance of CTLA-4 in the control of T-cell-mediated immunity has been demonstrated in knockout animals, where CTLA-4-deficient mice develop a fatal lymphoproliferative disorder characterized by rapid T-cell proliferation and extensive tissue damage, resulting in death at 4 weeks of age (12, 13).

CTLA-4 IN CANCER

It was hypothesized that CTLA-4 could inhibit T-cell-mediated antitumor immune responses by attenuating tumor-specific T-cell activation before these T-cells have been able to eradicate tumors, and that blockade of CTLA-4 would enhance T-cell-mediated antitumor immunity by removing this inhibitory signal. In mice, antibody-mediated blockade of CTLA-4 induces complete tumor rejection and immunologic memory in several murine models of cancer (14). In addition, preclinical murine studies have shown that CTLA-4 blockade synergizes with radiation therapy (15), chemotherapy (16), molecularly targeted therapy (17), and tumor vaccination (18) to eradicate established tumors. Mechanistic studies in mice have shown that CTLA-4 blockade increases the ratio of effector T-cells to Foxp3+ Treg cells in tumors (19). Blockade of CTLA-4 on Treg cells is critical to CTLA-4 blocking antibody therapy. CTLA-4 plays a major role in Treg-cell-mediated immunosuppression. Genetic ablation of CTLA-4 on Treg cells results in fatal autoimmunity and is sufficient to induce tumor regression in some models (20). In addition, maximal antitumor activity of CTLA-4 blockade requires engagement of CTLA-4 on both effector and Treg-cell populations (21). Furthermore, anti-CTLA-4 monoclonal antibodies of particular isotypes, such as IgG1, induce depletion of intra-tumoral Foxp3+ Treg cells through

antibody-dependent cell-mediated cytotoxicity by Fcγ receptor-expressing macrophages within the tumor microenvironment (22). This activity likely contributes to antitumor efficacy.

CTLA-4 BLOCKADE IN THE TREATMENT OF MELANOMA

Based on the promising antitumor activity of CTLA-4 inhibition in preclinical cancer models, several CTLA-4-blocking antibodies have been developed. Ipilimumab is a fully human monoclonal antibody of the IgG1 isotype that binds CTLA-4, preventing it from interacting with its ligands (23). Based on encouraging results in early clinical studies of ipilimumab for metastatic melanoma, ipilimumab was advanced into Phase III trials. In the first Phase III study, previously treated patients with unresectable Stage III or Stage IV melanoma were treated with ipilimumab alone, ipilimumab with a glycoprotein 100 (gp100) melanoma-specific peptide vaccine, or gp100 alone (24). This study demonstrated improved overall survival in patients receiving ipilimumab (10.1 months for ipilimumab alone and 10.0 months for ipilimumab and gp100, compared with 6.4 months for gp100 alone) and led to the FDA approval of ipilimumab for patients with late stage, unresectable melanoma. The overall response rate, including complete and partial responses, was 10.9% for ipilimumab, 5.7% for ipilimumab and gp100, and 1.5% for gp100 alone. A subsequent study demonstrated a median overall survival benefit of ipilimumab plus dacarbazine compared to placebo and dacarbazine (11.2 months vs. 9.1 months) in previously untreated metastatic melanoma patients (25). Overall response rates were 15.2% for ipilimumab and dacarbazine versus 10.3% for placebo and dacarbazine. In addition, ipilimumab therapy has demonstrated promising results in a Phase II study of melanoma patients with brain metastases, who have historically been a difficult patient population to treat (26). Pooled analysis of overall survival data of Phase II and Phase III trials including previously treated and treatment naïve advanced melanoma patients revealed a median overall survival of 11.4 months with a plateau in the survival curve at 22% at 3 years, demonstrating the durability of responses to ipilimumab (27). Ipilimumab is also efficacious in the adjuvant therapy of Stage III melanoma patients with pathological involvement of regional lymph nodes. In a Phase III study of Stage III melanoma patients who have undergone complete surgical resection, ipilimumab improved both the 5-year recurrence-free survival (40.8% vs. 30.3% with placebo) and the 5-year overall survival (65.4% vs. 54.4% with placebo) (28), resulting in the FDA approval for ipilimumab for the adjuvant therapy of melanoma.

Based upon preclinical studies discussed above, the mechanism of action of ipilimumab is enhancing T-cell-mediated antitumor immunity through blocking an inhibitory receptor on effector T-cells and depleting Treg cells. Analysis of pre- and posttreatment TCR expression from melanoma patients reveals that ipilimumab treatment leads to the expansion of T-cell clones not detected before therapy and only rarely boosts the expansion of T-cell clones present before therapy (29). In this way, ipilimumab is thought to broaden the repertoire of responding melanoma-specific T-cells. In addition, IFN-γ is central to the antitumor activity in CTLA-4 blockade, and anti-CTLA-4 treatment increases IFN-γ production by T-cells in both mouse models and patients (30, 31).

A second CTLA-4-blocking antibody, tremelimumab, has been developed. Tremelimumab is a fully human anti-CTLA-4 monoclonal antibody of the IgG2 isotype. Despite promising early clinical data in melanoma, tremelimumab failed to hit its primary endpoint of improved overall survival in comparison to standard of care chemotherapy for patients with previously untreated, unresectable Stage III or Stage IV melanoma (32). As a result, clinical development for melanoma was halted, but evaluation of tremelimumab in other cancers is currently ongoing.

TOXICITY OF CTLA-4 BLOCKADE

Given the ability of ipilimumab to enhance T-cell responses, ipilimumab treatment is associated with mechanism-based, immune-related adverse events. An early Phase II dosing study demonstrated a dose-dependent increase in immune-related adverse events with increasing ipilimumab dose (18% Grade 3 [severe] or Grade 4 [life-threatening] immune-related adverse events at 10 mg/kg vs. 5% Grade 3 or Grade 4 immune-related adverse events at 3 mg/kg) (33). Subsequent Phase III trials evaluated ipilimumab doses of 3 mg/kg and 10 mg/kg. The FDA-approved dose for melanoma treatment is 3 mg/kg every 3 weeks for four doses. In clinical trials, additional doses were given for stable disease or objective response. In the initial Phase III trial of ipilimumab at 3 mg/kg in patients with advanced melanoma, all immune-related adverse events developed during the induction and reinduction periods (24). Immune-related adverse events were generally reversible when managed with vigilant monitoring and systemic corticosteroids, as documented in the Risk Evaluation and Mitigation Strategy associated with the FDA approval. In the initial Phase III study of ipilimumab treatment of advanced melanoma, 17.4–22.9% of patients receiving ipilimumab experienced Grade 3 or Grade 4 treatment-related adverse events, with 10.2–14.5% of patients experiencing Grade 3 or Grade 4 immune-related adverse events. In addition, there were 14 treatment-related deaths, 7 of which were associated with immune-related adverse events. The most common sites for immune-related adverse events were the gastrointestinal tract and skin; 5.5–7.6% of ipilimumab-treated patients experienced Grade 3 or Grade 4 gastrointestinal immune-related adverse events, including diarrhea and colitis, and 1.5–2.3% of ipilimumab-treated patients had Grade 3 or Grade 4 skin immune-related adverse events, including pruritus, dermatitis, and vitiligo. Less frequently, patients experienced immune-related adverse events involving the endocrine system (hypothyroidism, hypopituitarism, hypophysitis, and adrenal insufficiency) or liver (hepatitis). Deaths associated with immune-related adverse events were a result of septicemia, bowel perforation, liver or multi-organ failure, or Guillain–Barre syndrome. In the second Phase III trial of ipilimumab treatment of advanced melanoma, treatment-related Grade 3 or Grade 4 adverse events occurring in 56.3% (41.7% due to immune-related adverse events) of patients receiving ipilimumab (10 mg/kg) and dacarbazine were increased compared with 27.5% (6% grade due to immune-related adverse events) of patients receiving dacarbazine and placebo (25). The FDA-approved dosing for the adjuvant therapy of melanoma is 10 mg/kg every 3 weeks for four doses followed by 10 mg/kg every 12 weeks for up to 3 years or until documented disease recurrence or unacceptable toxicity. In the Phase III trial of ipilimumab (10 mg/kg) in the adjuvant setting, there was an increased rate of

adverse events in patients receiving ipilimumab versus placebo. Adverse events of Grade 3 or Grade 4 occurred in 54.1% (41.6% due to immune-related adverse events) and Grade 5 (death) occurred in 1.1% of patients receiving ipilimumab compared with 26.2% Grade 3 or Grade 4 (2.7% due to immune-related adverse events) in patients receiving placebo (28). The incidence of immune-related adverse events in this study of the adjuvant setting was higher than that observed with the same dose in pooled analysis involving the treatment of patients with advanced melanoma, and 40% of patients discontinued adjuvant therapy. Of note, systemic immunosuppression for the management of immune-related adverse events does not impact on antitumor activity, suggesting that the immune-related mechanisms responsible for these autoimmune side effects are uncoupled from the antitumor immune response (34). Table 1 summarizes the clinical efficacy and adverse events with ipilimumab in the treatment of advanced melanoma.

Biology of PD-1

Programmed cell death protein 1 (PD-1) is another immune checkpoint in the Ig superfamily (35, 36). Like CTLA-4, PD-1 inhibits T-cell activity and is expressed by activated T-cells (Figure 1). However, instead of competitively inhibiting co-stimulation by interfering with CD28/B7 ligand interaction, PD-1 negatively regulates TCR-signaling events. While CTLA-4 inhibits T-cells during the priming phase of immune responses, PD-1 is thought to inhibit activated T-cells at a later stage in peripheral tissues. In this way, PD-1 plays a critical role in the maintenance of peripheral T-cell tolerance. Consistent with the role of PD-1 in the prevention of autoimmunity, PD-1-deficient mice spontaneously develop late-onset autoimmunity, including lupus-like arthritis, glomerulonephritis, and cardiomyopathy, which is less severe, less frequent, and occurs later in life than CTLA-4-deficient mice (35–38).

PD-1 expression is absent on resting T-cells and is upregulated following activation (39, 40). Persistent T-cell stimulation, as present during chronic viral infection and cancer, induces high levels of PD-1 expression, which subsequently induces a state of T-cell exhaustion where T-cells gradually lose effector functions. PD-1 has two ligands, namely, PD-L1 (35, 41) and PD-L2 (42). PD-L1 is constitutively expressed on a variety of immune cells, including T-cells, B-cells, dendritic cells (DC), NK cells, monocytes, and macrophages (43), as well as a number of nonhematopoietic cells, including vascular endothelial cells (44) and many tumor cells (45). PD-L1 expression can also be upregulated by pro-inflammatory cytokines such as IFN-γ (44, 46). PD-L2 is expressed on APC and can be induced on tumor cells, including ~2% of melanoma cases (47).

The mechanism by which PD-1 inhibits T-cell activation is distinct from CTLA-4. The intracellular region of the PD-1 protein contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM) that play critical roles in PD-1-mediated suppression (48). Binding of PD-1 to its ligands triggers the phosphorylation of its ITIM and ITSM domains which subsequently induces the recruitment of Src homology region 2 domain-containing phosphatase-1 (SHP-1) and phosphatase-2 (SHP-2) (48, 49).

TABLE 1 Ipilimumab Clinical Data in Advanced Melanoma Treatment

Study arms	n	Previous treatment	Ipilimumab dose	1-Year overall survival (%)	Median overall survival	Objective response rate	Grade 3–Grade 4 AE (%)	Reference
Ipilimumab	131	Yes	3 mg/kg q3wks × 4 doses*	45.6	10.1 months**	10.9%	22.9***	24
Ipilimumab + gp100	380			43.6	10.0 months**	5.7%	17.4***	
gp100 peptide vaccine	132			25.3	6.4 months	1.5%	11.4***	
Ipilimumab + Dacarbazine	250	No	10 mg/kg q3wks × 4 doses*	47.3**	11.2 months**	15.2%	56.3**	25
Placebo + dacarbazine	252			36.3	9.1 months	10.3%	27.5	
Pooled analysis of overall survival data of Phase II and III trials	1861	Mixture	3 mg/kg 10 mg/kg × 4 doses*		11.4 months 11.1 months	3-year survival rate: 22% with plateau		27

AE, treatment-related adverse event; q3wks, every 1 weeks; *additional doses for stable disease or objective response; **statistically significant difference; ***in addition there were four deaths in ipilimumab alone group, eight deaths in ipilimumab + gp100 group, and two deaths in gp100 alone group.

These phosphatases then dephosphorylate members of the TCR-signaling complex, resulting in the inhibition of T-cell activation. Signaling through PD-1 inhibits TCR-induced proliferation, cytokine secretion, and expression of the pro-survival gene, Bcl-xL (35, 39).

PD-1 IN CANCER

The PD-1/PD-L1 axis represents a critical immune escape mechanism for cancer. In murine models of melanoma, PD-L1 expression correlates with diminished antitumor CD8+ T-cell activity, and antitumor T-cell activity can be restored by genetic deletion of PD-1 on T-cells or by treatment with PD-L1-blocking antibodies (50). Tumor-specific T-cell populations from melanoma patients often express high levels of PD-1, and melanoma tumor-infiltrating CD8+ T-cells often display functional impairment consistent with exhaustion (51–55). Elevated PD-L1 expression has been observed on both tumor cells and immune cell infiltrates in many different cancers, including melanoma (46, 56–59). Expression of PD-L1 in melanoma is associated with immune cell infiltration of tumors. PD-L1 expression is often located in close proximity to CD8+ T-cell infiltrates, and IFN-γ produced by these lymphocytes can lead to the upregulation of PD-L1 expression (56, 58, 59). These findings suggest that PD-1/PD-L1 functions as an adaptive tumor immune escape mechanism and that infiltrating T-cells may induce their own suppression through the production of pro-inflammatory cytokines. In a mouse model of chronic infection, anti-PD-L1 antibody treatment reinvigorates exhausted T-cells, but only produces minimal memory, and T-cells reacquire the exhausted phenotype with persistent antigen, suggesting a limited duration of antitumor T-cell responses to blockade of the PD-1/PD-L1 axis (60).

In addition to the well-established role of PD-1 on T-cells, a recent study demonstrates that PD-1 has an intrinsic effect in melanoma cells (61). A portion of human melanoma cells express PD-1. In *in vitro* studies, mouse models and human xenografts, PD-1 expression on melanoma cells promotes tumor growth, and inhibition of PD-1 reduces melanoma growth independent of the adaptive immune system. Furthermore, anti-PD1 treatment in human melanoma patients is associated with diminished PD-1 receptor signaling in melanoma cells, and a high frequency of PD-1 receptor signaling in melanoma cells pretreatment is associated with improved progression-free survival (PFS).

PD-1 BLOCKADE IN THE TREATMENT OF MELANOMA

Based on preclinical animal studies showing that blockade of the PD-1/PD-L1 signaling axis can restore the function of exhausted T-cells to mediate antitumor immunity, several PD-1-blocking antibodies have been developed, including nivolumab and pembrolizumab. PD-L1 blockade is also being explored. Although antibodies blocking PD-L1 have been FDA-approved in the treatment of urothelial carcinoma, nonsmall cell lung cancer and Merkel cell carcinoma, to date no anti-PD-L1 antibodies have received FDA approval in melanoma.

Nivolumab is a fully human monoclonal antibody of the IgG4 isotype that binds to PD-1, preventing it from interacting with its ligands. Early clinical studies of nivolumab showed promising antitumor activity against a variety of tumor

types, including melanoma. Based on these results, Phase III trials were initiated to test nivolumab against standard of care chemotherapy, first in previously treated patients and then as a first-line treatment. In a Phase III trial enrolling Stage III or Stage IV melanoma patients who had failed prior ipilimumab or BRAF inhibitor therapy, nivolumab demonstrated activity in patients with and without BRAF mutations and had an objective response rate of 31.7% compared to 10.7% for patients on chemotherapy (62). In another Phase III trial, nivolumab was tested in treatment-naïve melanoma patients with wild-type BRAF (63). In this study, patients were treated with either nivolumab or dacarbazine, and the nivolumab group demonstrated improved efficacy in terms of 1-year overall survival (72.9% vs. 42.1%), median PFS (5.1 months vs. 2.2 months), and objective response rate (40.0% vs. 13.9%).

CTLA-4 and PD-1 induce T-cell suppression through nonoverlapping mechanisms and likely impact different populations of T-cells during different phases of the immune response (CTLA-4 during priming and PD-1 during the effector phase), providing a mechanistic rationale for the combination of CTLA-4 and PD-1 blockade. A subsequent Phase III trial in previously untreated melanoma patients compared nivolumab and ipilimumab combination therapy, nivolumab alone, and ipilimumab alone (64). The median PFS was 11.5 months, 6.9 months, and 2.9 months, respectively. The objective response rate was 57.6, 43.7, and 19.0%, respectively. The median PFS and the objective response rate were significantly improved in both the nivolumab and ipilimumab combination and the nivolumab alone groups compared with the ipilimumab group. Based on these studies, nivolumab is FDA-approved as a monotherapy in advanced melanoma patients with wild-type BRAF and received accelerated approval for monotherapy in patients with $BRAF^{V600E}$ mutation and in combination with ipilimumab.

A second anti-PD-1-blocking antibody was developed called pembrolizumab. Like nivolumab, pembrolizumab is a fully human monoclonal antibody of the IgG4 isotype that binds to human PD-1 preventing ligand interaction. A Phase II trial of advanced melanoma patients, who progressed on ipilimumab therapy or BRAF and/or MEK inhibitors, demonstrated improved PFS with pembrolizumab at both 2 mg/kg and 10 mg/kg doses every 3 weeks compared with investigators' choice of chemotherapy (65). A randomized Phase III trial compared pembrolizumab every 2 weeks, pembrolizumab every 3 weeks, and ipilimumab in the first-line treatment of advanced melanoma (66). Pembrolizumab every 2 weeks and every 3 weeks demonstrated improved efficacy compared with ipilimumab, in terms of 1-year overall survival (74.1 and 68.4% vs. 58.2%), median PFS (5.5 months and 4.1 months vs. 2.8 months), and objective response rate (33.7 and 32.9% vs. 11.9%). Based on these studies, pembrolizumab at 2 mg/kg every 3 weeks is FDA-approved for the treatment of advanced melanoma.

Toxicity of PD-1 blockade

The most common adverse events observed following PD-1 blockade are fatigue, rash, diarrhea, pruritus, and nausea (62–64, 66). A similar pattern of mechanism-based, immune-related adverse events are seen with PD-1 blockade as with CTLA-4 blockade with ipilimumab. The vast majority of Grade 3 and Grade 4

immune-related adverse events resolve quickly with delay in treatment and/or administration of systemic corticosteroids using established safety management guidelines (62, 63). Consistent with mouse studies in which the autoimmune pathology of PD-1-deficient mice is decreased in severity compared to CTLA-4-deficient mice, the toxicity associated with PD-1 blockade is diminished in comparison to CTLA-4 blockade. In a Phase III trial of head to head comparison of pembrolizumab and ipilimumab, both pembrolizumab groups had a significantly lower incidence of Grade 3–Grade 5 adverse events compared with ipilimumab (10.1–13.3% in the pembrolizumab groups vs. 19.9% in the ipilimumab group), despite an approximately 3-fold longer duration of pembrolizumab therapy (66). Although the combination of ipilimumab and nivolumab therapy resulted in improved clinical efficacy, the combination therapy group had a higher incidence of Grade 3 and Grade 4 adverse events compared with either nivolumab or ipilimumab alone (55.5% vs. 16.3% or 27.3%, respectively) (64). Table 2 summarizes the clinical efficacy and adverse events of Phase III trials of PD-1 blockade as a first-line treatment of advanced melanoma.

Taken together, PD-1 blockade has become the first-line therapy for advanced melanoma patients, given its improved clinical efficacy and improved safety profile compared with ipilimumab. It remains to be determined whether PD-1 blockade results in the same long-term duration of response as ipilimumab.

Biomarkers of Checkpoint Inhibitor Activity

The clinical development of CTLA-4 and PD-1-/PD-L1-blocking antibodies has had a profound impact on the treatment of melanoma and several other cancers. However, despite this success, only a minority of advanced melanoma patients respond to checkpoint blockade, with a 10–40% objective response rate with monotherapy and up to 58% with combined ipilimumab and nivolumab. As a result, considerable effort is being invested in the identification of predictive biomarkers to identify patients most likely to benefit from checkpoint blockade and those at high risk for treatment failure who would benefit from more aggressive combination therapy in order to limit unnecessary exposure to immune-related adverse events. Early clinical experience with immune checkpoint blockade has identified several biomarkers associated with treatment efficacy, including tumor mutational burden, the presence of tumor-infiltrating lymphocytes, PD-L1 expression, and intestinal microbiota.

The primary mechanism of action of checkpoint inhibitor therapy involves the activation of antitumor T-cells. Many of the tumor-specific T-cells recognize tumor expressed “neoantigens” that are a product of mutational events in tumor cells (67, 68). Since these mutations arise through a random process, it is thought that tumors characterized by a high overall mutational load are more likely to result in the formation of immunogenic neoantigens. Whole exome sequencing of melanoma patients treated with anti-CTLA-4 therapy revealed that antitumor responses were associated with high mutational load, and strong responders expressed a specific antigen signature (69). Similarly, neoantigen load was shown to correlate with clinical response in a second cohort of melanoma patients treated with ipilimumab (70). The clonality of neoantigen expression has also been correlated with

TABLE 2 PD-1 Blockade Clinical Data in the First-Line Treatment of Advanced Melanoma

Study arms	n	Dose and frequency	1-year overall survival (%)	Median PFS	Objective response rate (%)	Grade 3–Grade 4 AE (%)	Reference
Nivolumab	206	3 mg/kg q2wks	72.9*	5.1 months*	40.0*	11.7*	63
Dacarbazine	205		42.1	2.2 months	13.9	17.6	
Nivolumab + Ipilimumab	314	1 mg/kg q3wks × 4 then 3 mg/kg q2wks 3 mg/kg q3wks × 4 doses	N/A	11.5 months*	57.6*	55.0*	64
Nivolumab	316	3 mg/kg q2wks		6.9 months*	43.7*	16.3**	
Ipilimumab	315	3 mg/kg q3wks × 4 doses		2.9 months	19.0	27.3**	
Pembrolizumab	278	10 mg/kg q2wks	74.1*	5.5 months*	33.7*	13.3*	66
Pembrolizumab	277	10 mg/kg q3wks	68.4*	4.1 months*	32.9*	10.1*	
Ipilimumab	256	3 mg/kg q3wks × 4 doses	58.2	2.8 months	11.9	19.5**	

AE, treatment-related adverse events; N/A, not available; PFS, progression-free survival; q2wks, every 2 weeks; q3wks, every 3 weeks *statistically significant difference; **in addition there was one patient death.

clinical response to checkpoint inhibitors. Melanoma and nonsmall cell lung cancer patients with neoantigens expressed in all tumor cells (clonal neoantigens) experienced long-term clinical benefit to anti-CTLA-4 and anti-PD-1 treatment (71). However, patients with neoantigens expressed in only a subset of their tumor cells (subclonal neoantigens) responded poorly to checkpoint blockade (71).

Preexisting tumor-infiltrating lymphocytes have been associated with clinical responses to PD-1 blockade. In melanoma, response to pembrolizumab is associated with a higher number of CD8+, PD-1+, and PD-L1+ cells within the tumor and at the invasive margin at baseline; the proximity of PD-1+ and PD-L1+ cells at baseline; and an increased density and proliferation of CD8+ T-cells on treatment, suggesting the need for preexisting T-cells in the tumor inhibited by PD-1/PD-L1 interaction (59). Flow cytometric analysis of melanoma tissue biopsies from patients undergoing treatment with pembrolizumab also showed that patients who responded to therapy had increased frequencies of tumor-infiltrating CD8+ memory T-cells compared to nonresponders (72).

Although early clinical trials showed an association between PD-L1 expression and objective response in patients with metastatic melanoma treated with PD-1 or PD-L1 blockade, patients whose tumor cells lacked PD-L1 expression still benefited from PD-1/PD-L1 blockade. Phase III trials of PD-1 blockade have demonstrated similar results. Although the subgroup of patients with tumor cells that were PD-L1+ had numerically higher objective response rates, patient subgroups with tumor cells that were PD-L1+ and PD-L1– both demonstrated improved overall survival and objective response rates when treated with nivolumab compared with dacarbazine (63). An overall survival benefit of pembrolizumab compared with ipilimumab was not observed in the subgroup of patients with PD-L1– tumor cells; however, the sample size of PD-L1– patients was too small to draw definitive conclusions (66). The patient subgroup with PD-L1+ tumor cells had the same PFS with combination ipilimumab and nivolumab therapy as with nivolumab alone, whereas PFS in the subgroup with PD-L1– tumor cells was improved with combination therapy versus nivolumab alone, suggesting that patients with PD-L1– tumor may have greater benefit from combination therapy (64). Yet, 41.3% of patients with PD-L1– tumors had an objective response to nivolumab alone (64). Analysis and interpretation of PD-L1 expression in tumors is complicated by multiple factors. Different trials used different anti-PD-L1 antibodies and immunohistochemical assays and different cutoff points for defining PD-L1 positivity. While most studies have assessed PD-L1 expression on tumor cells, PD-L1 expression on T-cells and macrophages may influence response to PD-1 blockade (57, 73). PD-L1 expression measured at one time point and in one metastasis is not representative, as PD-L1 expression is dynamic and differs in different metastases from the same patient (57, 66). Lastly, other PD-1 ligands may be involved in response to PD-1 blockade. In summary, clinical experience to date indicates that lack of PD-L1 expression in tumor cells is not a reason to withhold anti-PD-1 therapy.

In murine models, the presence of certain species of intestinal bacteria is associated with spontaneous antitumor immunity, and the presence of these bacteria can improve responses to CTLA-4 and PD-1 blockade (74, 75). In addition, T-cells, specific for some of these bacteria, are found in melanoma patients responding to anti-CTLA-4 treatment (75). The mechanism by which intestinal microbiota modulate antitumor immune responses is thought to involve the

activation of innate immune cells, including DC, making them better able to stimulate T-cells. Alternatively, specific antigens from these bacteria may mimic antigens expressed by the tumor, leading to the activation of tumor cross-reactive T-cells.

As antigen load is a key factor in T-cell exhaustion in preclinical models, more integrated strategies to predict the response to therapy are under investigation. These strategies incorporate immune status and tumor burden. A recent study found that the magnitude of reinvigorated, exhausted CD8+ T-cells in the peripheral blood on treatment with pembrolizumab in relationship to the pretreatment tumor burden correlated with clinical response, suggesting a clinically accessible on-treatment predictor of response (76). A more comprehensive strategy called the "cancer immunogram" incorporating the tumor mutational load, general immune status of the patient, immune cell infiltration of the tumor, absence of checkpoints, absence of soluble inhibitors, absence of inhibitory metabolism, and sensitivity to immune effectors is also under development (77).

Mechanisms of Resistance to Immune Checkpoint Blockade

Recent clinical experience has uncovered several resistance mechanisms to immune checkpoint blockade. These resistance mechanisms involve changes to the tumor microenvironment that limit T-cell activation, tumor infiltration, and effector-mediated destruction of tumor cells. A lack of tumor-associated antigens can impair tumor-specific T-cell activation and allows tumors to escape immune checkpoint blockade. Failure of tumor antigen presentation can occur as a result of outright antigen loss or from defects in components of antigen processing and presentation pathways. Failure of tumor antigen presentation is a major mechanism by which tumors escape from T-cell-mediated immune recognition (78, 79). Analysis of pretreatment and posttreatment tumor samples from patients with nonsmall cell lung cancer treated with checkpoint blockade revealed the loss of several neoantigens from treatment-refractory tumor cell clones (80). These neoantigens were capable of stimulating T-cell responses *in vitro*, and their loss coincided with the emergence of disease resistance. Mutations in β2-microglobulin, a protein required for the folding and transport of MHC Class I to the cell surface, have also been observed in melanoma patients at the time of anti-PD-1 treatment failure (81).

Mechanisms that inhibit T-cell trafficking to tumor tissue also cause resistance to immune checkpoint inhibitors. Mutations in BRAF and loss of PTEN expression both contribute to immune checkpoint blockade resistance in murine models and patients by inducing the production of a number of immunosuppressive proteins, including VEGF that limits T-cell trafficking to tumor sites and inhibits T-cell effector functions (82, 83). In addition, melanoma patients whose tumors had elevated signaling activity in the WNT/β-catenin pathway lacked infiltrating T-cells, and murine studies have shown that WNT signaling can promote anti-PD-L1/anti-CTLA-4 treatment failure in melanoma models (84).

Mutations in genes involved in the IFN-γ signaling pathway also contribute to both primary and acquired resistance to immune checkpoint blockade.

IFN-γ signaling plays a critical role in T-cell-mediated antitumor immunity by enhancing MHC expression and subsequent tumor antigen presentation, inducing the recruitment of other immune cells, inhibiting tumor cell proliferation, and inducing tumor cell apoptosis (85). IFN-γ binds to the interferon gamma receptor 1 (IFNGR1) and interferon gamma receptor 2 (IFNGR2) and signals through the Janus-activated kinase 1 (JAK1) and Janus-activated kinase 2 (JAK2)/signal transducer and activator of transcription 1 (STAT1) signaling pathway (85). Ipilimumab-refractory melanoma tumors were insensitive to IFN-γ signaling due to mutations in IFNGR1, IFNGR2, JAK2, and interferon regulatory factor 1 (IRF1) which is responsible for the INF-γ-induced upregulation of PD-L1 (86). Mutations in JAK1 and JAK2 were also found in melanoma and colorectal cancer patients who failed to respond to anti-PD1 despite having tumors with high mutational load (87, 88). Similar mutations in JAK1 and JAK2 were also detected in relapsing tumors from melanoma patients who initially responded to anti-PD-1 therapy, indicating that loss of responsiveness to IFN-γ signaling may be a potential tumor escape mechanism contributing to relapse following immune checkpoint blockade (81).

Tumor-extrinsic mechanisms of resistance to immune checkpoint blockade have also been identified, including additional immune checkpoint receptors, immunosuppressive cytokines, and other factors present in the tumor microenvironment and immunosuppressive immune cell populations. In addition to CTLA-4 and PD-1, several other immune checkpoint receptors have been identified including lymphocyte activation gene 3 (LAG-3), T-cell immunoglobulin and mucin 3 (TIM-3), V-domain Ig-containing suppressor of T-cell activation (VISTA), and T-cell immunoreceptor with Ig and ITIM domains (TIGIT) that are expressed by T-cells and negatively regulate immune responses (89). These checkpoints are often co-expressed by CTLA-4 and PD-1-expressing T-cells within tumors, and their expression can be upregulated following anti-CTLA-4 and anti-PD-1 treatment (89).

Immunosuppressive factors in the tumor microenvironment produced by tumor cells and infiltrating immune cells may also cause immune checkpoint blockade resistance by inhibiting T-cell activity. TGF-β is an immunosuppressive cytokine produced by many different human tumor types that may limit the efficacy of checkpoint blockade by stimulating Treg cells and impairing T-cell function (90). In addition, indoleamine-pyrrole 2,3-dioxygenase (IDO), an enzyme responsible for the breakdown of tryptophan, is expressed in many tumors and may inhibit T-cell proliferation by depleting tryptophan (89). CD73, an ecto-enzyme responsible for mediating the catalysis of adenosine monophosphate to adenosine, is expressed by many tumors and is associated with anti-PD-1 resistance in murine models (91). Elevated levels of adenosine, as a result of CD73 expression, suppress T-cell activity by signaling through the adenosine receptor 2A (91).

Certain tumor-infiltrating immune cell populations also contribute to immune checkpoint blockade resistance. Myeloid-derived suppressor cells (MDSCs) are a CD11b+CD33+ myeloid cell population that plays an immunoregulatory role in a number of disease states, including cancer (92). MDSCs are immunosuppressive and contribute to angiogenesis, tumor invasion, and metastasis (93, 94). In addition, pretreatment MDSC frequencies are inversely correlated with clinical responses to ipilimumab and nivolumab in melanoma patients (95, 96).

Future Directions

These resistance mechanisms must be overcome in order to improve the clinical efficacy of immune checkpoint blockade for melanoma and other cancers. A number of strategies are currently being tested to target additional sources of immunosuppression in the tumor microenvironment for use in combination with immune checkpoint inhibitors. Tumor-specific peptide and cell-based vaccines are being tested in combination with CTLA-4 and PD-1-/PD-L1-blocking antibodies in order to boost antitumor T-cell responses (97). Molecularly targeted agents are also being combined with immune checkpoint inhibitors. BRAF inhibition, which is FDA-approved for the treatment of metastatic melanoma expressing the activating $BRAF^{V600E}$ mutation, has been shown to increase MHC expression, tumor antigen presentation, and T-cell infiltration (98–102). Similarly, MEK inhibitors have been shown to improve CD8+ T-cell activity in preclinical models in combination with PD-1 blockade (103). Given the clinical success of CTLA-4 and PD-1/PD-L1 inhibition, blocking antibodies have been developed to target additional immune checkpoints, including LAG-3, TIM-3, VISTA, and TIGIT, and these agents have entered clinical trials alone and in combination with anti-CTLA-4 and anti-PD-1/PD-L1 (89, 97). Furthermore, numerous treatments are being developed to target immunosuppressive cytokines and other factors present in the tumor microenvironment, including IDO inhibitors, CD73 blocking antibodies, and adenosine receptor 2A antagonists (97). Finally, strategies to deplete or reprogram MDSC are also under development for use in combination with immune checkpoint blockade. Signaling through the gamma isoform of phosphatidylinositide 3-kinase (PI3Kγ) is critical for the maintenance of myeloid cell immunosuppression in tumors, and genetic deletion or pharmacologic inhibition of PI3Kγ results in tumor-infiltrating myeloid cells with a more pro-inflammatory phenotype in murine models (104). In addition, the small molecule PI3Kγ inhibitor IPI-549 improved the ability of immune checkpoint blockade to induce tumor regression in preclinical murine models of melanoma, breast, and head and neck cancer (104, 105).

Conclusion

Immune checkpoint inhibitors have revolutionized the treatment of melanoma and many other cancers. Blocking antibodies to CTLA-4 and PD-1/PD-L1 have improved survival for many patients, and long-term durable responses have been observed in some patients. However, despite this promise, clinical benefit from immune checkpoint blockade is only seen in a minority of melanoma patients, and autoimmune toxicity, while manageable, requires careful monitoring. Clinical experience with anti-CTLA-4 and anti-PD-1/PD-L1 therapy has uncovered critical parameters that govern effective antitumor immune responses. This knowledge is leading to the identification of subsets of patients most likely to respond to therapy with immune checkpoint inhibitors. In addition, these insights have identified new immune targets that promise to expand the clinical reach of immunotherapy to more patients and cancer types.

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References

1. Callahan MK, Postow MA, Wolchok JD. Targeting T Cell co-receptors for cancer therapy. Immunity. 2016 May 17;44(5):1069–78. http://dx.doi.org/10.1016/j.immuni.2016.04.023
2. Fife BT, Bluestone JA. Control of peripheral T-cell tolerance and autoimmunity via the CTLA-4 and PD-1 pathways. Immunol Rev. 2008 Aug;224:166–82. http://dx.doi.org/10.1111/j.1600-065X.2008.00662.x
3. Brunet JF, Denizot F, Luciani MF, Roux-Dosseto M, Suzan M, Mattei MG, et al. A new member of the immunoglobulin superfamily--CTLA-4. Nature. 1987 Jul 16–22;328(6127):267–70. http://dx.doi.org/10.1038/328267a0
4. Krummel MF, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. J Exp Med. 1995 Aug 01;182(2):459–65. http://dx.doi.org/10.1084/jem.182.2.459
5. Krummel MF, Allison JP. CTLA-4 engagement inhibits IL-2 accumulation and cell cycle progression upon activation of resting T cells. J Exp Med. 1996 Jun 01;183(6):2533–40. http://dx.doi.org/10.1084/jem.183.6.2533
6. Walunas TL, Lenschow DJ, Bakker CY, Linsley PS, Freeman GJ, Green JM, et al. CTLA-4 can function as a negative regulator of T cell activation. Immunity. 1994 Aug;1(5):405–13. http://dx.doi.org/10.1016/1074-7613(94)90071-X
7. Linsley PS, Brady W, Urnes M, Grosmaire LS, Damle NK, Ledbetter JA. CTLA-4 is a second receptor for the B cell activation antigen B7. J Exp Med. 1991 Sep 01;174(3):561–9. http://dx.doi.org/10.1084/jem.174.3.561
8. Linsley PS, Greene JL, Brady W, Bajorath J, Ledbetter JA, Peach R. Human B7-1 (CD80) and B7-2 (CD86) bind with similar avidities but distinct kinetics to CD28 and CTLA-4 receptors. Immunity. 1994 Dec;1(9):793–801. http://dx.doi.org/10.1016/S1074-7613(94)80021-9
9. Qureshi OS, Zheng Y, Nakamura K, Attridge K, Manzotti C, Schmidt EM, et al. Trans-endocytosis of CD80 and CD86: A molecular basis for the cell-extrinsic function of CTLA-4. Science. 2011 Apr 29;332(6029):600–3. http://dx.doi.org/10.1126/science.1202947
10. Lindsten T, Lee KP, Harris ES, Petryniak B, Craighead N, Reynolds PJ, et al. Characterization of CTLA-4 structure and expression on human T cells. J Immunol. 1993 Oct 01;151(7):3489–99.
11. Egen JG, Allison JP. Cytotoxic T lymphocyte antigen-4 accumulation in the immunological synapse is regulated by TCR signal strength. Immunity. 2002 Jan;16(1):23–35. http://dx.doi.org/10.1016/S1074-7613(01)00259-X
12. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunity. 1995 Nov;3(5):541–7. http://dx.doi.org/10.1016/1074-7613(95)90125-6

13. Waterhouse P, Penninger JM, Timms E, Wakeham A, Shahinian A, Lee KP, et al. Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. Science. 1995 Nov 10;270(5238):985–8. http://dx.doi.org/10.1126/science.270.5238.985
14. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science. 1996 Mar 22;271(5256):1734–6. http://dx.doi.org/10.1126/science.271.5256.1734
15. Tang C, Wang X, Soh H, Seyedin S, Cortez MA, Krishnan S, et al. Combining radiation and immunotherapy: A new systemic therapy for solid tumors? Cancer Immunol Res. 2014 Sep;2(9):831–8. http://dx.doi.org/10.1158/2326-6066.CIR-14-0069
16. Jure-Kunkel M, Masters G, Girit E, Dito G, Lee F, Hunt JT, et al. Synergy between chemotherapeutic agents and CTLA-4 blockade in preclinical tumor models. Cancer Immunol Immunother. 2013 Sep;62(9):1533–45.
17. Hu-Lieskovan S, Mok S, Homet Moreno B, Tsoi J, Robert L, Goedert L, et al. Improved antitumor activity of immunotherapy with BRAF and MEK inhibitors in BRAF(V600E) melanoma. Sci Transl Med. 2015 Mar 18;7(279):279ra41. http://dx.doi.org/10.1126/scitranslmed.aaa4691
18. van Elsas A, Hurwitz AA, Allison JP. Combination immunotherapy of B16 melanoma using anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing vaccines induces rejection of subcutaneous and metastatic tumors accompanied by autoimmune depigmentation. J Exp Med. 1999 Aug 02;190(3):355–66. http://dx.doi.org/10.1084/jem.190.3.355
19. Quezada SA, Peggs KS, Curran MA, Allison JP. CTLA4 blockade and GM-CSF combination immunotherapy alters the intratumor balance of effector and regulatory T cells. J Clin Invest. 2006 Jul;116(7):1935–45. http://dx.doi.org/10.1172/JCI27745
20. Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, et al. CTLA-4 control over Foxp3+ regulatory T cell function. Science. 2008 Oct 10;322(5899):271–5. http://dx.doi.org/10.1126/science.1160062
21. Peggs KS, Quezada SA, Chambers CA, Korman AJ, Allison JP. Blockade of CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumor activity of anti-CTLA-4 antibodies. J Exp Med. 2009 Aug 03;206(8):1717–25. http://dx.doi.org/10.1084/jem.20082492
22. Simpson TR, Li F, Montalvo-Ortiz W, Sepulveda MA, Bergerhoff K, Arce F, et al. Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma. J Exp Med. 2013 Aug 26;210(9):1695–710. http://dx.doi.org/10.1084/jem.20130579
23. Keler T, Halk E, Vitale L, O'Neill T, Blanset D, Lee S, et al. Activity and safety of CTLA-4 blockade combined with vaccines in cynomolgus macaques. J Immunol. 2003 Dec 01;171(11):6251–9. http://dx.doi.org/10.4049/jimmunol.171.11.6251
24. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010 Aug 19;363(8):711–23. http://dx.doi.org/10.1056/NEJMoa1003466
25. Robert C, Thomas L, Bondarenko I, O'Day S, Weber J, Garbe C, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med. 2011 Jun 30;364(26):2517–26. http://dx.doi.org/10.1056/NEJMoa1104621
26. Margolin K, Ernstoff MS, Hamid O, Lawrence D, McDermott D, Puzanov I, et al. Ipilimumab in patients with melanoma and brain metastases: An open-label, phase 2 trial. Lancet Oncol. 2012 May;13(5):459–65. http://dx.doi.org/10.1016/S1470-2045(12)70090-6
27. Schadendorf D, Hodi FS, Robert C, Weber JS, Margolin K, Hamid O, et al. Pooled analysis of long-term survival data from phase II and phase III trials of ipilimumab in unresectable or metastatic melanoma. J Clin Oncol. 2015 Jun 10;33(17):1889–94.
28. Eggermont AM, Chiarion-Sileni V, Grob JJ, Dummer R, Wolchok JD, Schmidt H, et al. Prolonged survival in stage III melanoma with ipilimumab adjuvant therapy. N Engl J Med. 2016 Nov 10;375(19):1845–55. http://dx.doi.org/10.1056/NEJMoa1611299
29. Kvistborg P, Philips D, Kelderman S, Hageman L, Ottensmeier C, Joseph-Pietras D, et al. Anti-CTLA-4 therapy broadens the melanoma-reactive CD8+ T cell response. Sci Transl Med. 2014 Sep 17;6(254):254ra128. http://dx.doi.org/10.1126/scitranslmed.3008918

30. Liakou CI, Kamat A, Tang DN, Chen H, Sun J, Troncoso P, et al. CTLA-4 blockade increases IFNgamma-producing CD4+ICOShi cells to shift the ratio of effector to regulatory T cells in cancer patients. Proc Natl Acad Sci U S A. 2008 Sep 30;105(39):14987–92. http://dx.doi.org/10.1073/pnas.0806075105
31. Shi LZ, Fu T, Guan B, Chen J, Blando JM, Allison JP, et al. Interdependent IL-7 and IFN-gamma signalling in T-cell controls tumour eradication by combined alpha-CTLA-4+alpha-PD-1 therapy. Nat Commun. 2016 Aug 08;7:12335. http://dx.doi.org/10.1038/ncomms12335
32. Ribas A, Kefford R, Marshall MA, Punt CJ, Haanen JB, Marmol M, et al. Phase III randomized clinical trial comparing tremelimumab with standard-of-care chemotherapy in patients with advanced melanoma. J Clin Oncol. 2013 Feb 10;31(5):616–22. http://dx.doi.org/10.1200/JCO.2012.44.6112
33. Wolchok JD, Neyns B, Linette G, Negrier S, Lutzky J, Thomas L, et al. Ipilimumab monotherapy in patients with pretreated advanced melanoma: A randomised, double-blind, multicentre, phase 2, dose-ranging study. Lancet Oncol. 2010 Feb;11(2):155–64. http://dx.doi.org/10.1016/S1470-2045(09)70334-1
34. Horvat TZ, Adel NG, Dang TO, Momtaz P, Postow MA, Callahan MK, et al. Immune-related adverse events, need for systemic immunosuppression, and effects on survival and time to treatment failure in patients with melanoma treated with ipilimumab at Memorial Sloan Kettering Cancer Center. J Clin Oncol. 2015 Oct 01;33(28):3193–8.
35. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med. 2000 Oct 02;192(7):1027–34. http://dx.doi.org/10.1084/jem.192.7.1027
36. Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J. 1992 Nov;11(11):3887–95.
37. Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. Immunity. 1999 Aug;11(2):141–51. http://dx.doi.org/10.1016/S1074-7613(00)80089-8
38. Nishimura H, Okazaki T, Tanaka Y, Nakatani K, Hara M, Matsumori A, et al. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. Science. 2001 Jan 12;291(5502):319–22. http://dx.doi.org/10.1126/science.291.5502.319
39. Chemnitz JM, Parry RV, Nichols KE, June CH, Riley JL. SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation. J Immunol. 2004 Jul 15;173(2):945–54. http://dx.doi.org/10.4049/jimmunol.173.2.945
40. Nishimura H, Agata Y, Kawasaki A, Sato M, Imamura S, Minato N, et al. Developmentally regulated expression of the PD-1 protein on the surface of double-negative (CD4-CD8-) thymocytes. Int Immunol. 1996 May;8(5):773–80. http://dx.doi.org/10.1093/intimm/8.5.773
41. Dong H, Zhu G, Tamada K, Chen L. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. Nat Med. 1999 Dec;5(12):1365–9. http://dx.doi.org/10.1038/70932
42. Latchman Y, Wood CR, Chernova T, Chaudhary D, Borde M, Chernova I, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. Nat Immunol. 2001 Mar;2(3):261–8. http://dx.doi.org/10.1038/85330
43. Yamazaki T, Akiba H, Iwai H, Matsuda H, Aoki M, Tanno Y, et al. Expression of programmed death 1 ligands by murine T cells and APC. J Immunol. 2002 Nov 15;169(10):5538–45. http://dx.doi.org/10.4049/jimmunol.169.10.5538
44. Eppihimer MJ, Gunn J, Freeman GJ, Greenfield EA, Chernova T, Erickson J, et al. Expression and regulation of the PD-L1 immunoinhibitory molecule on microvascular endothelial cells. Microcirculation. 2002 Apr;9(2):133–45. http://dx.doi.org/10.1080/713774061
45. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: A potential mechanism of immune evasion. Nat Med. 2002 Aug;8(8):793–800.
46. Schreiner B, Mitsdoerffer M, Kieseier BC, Chen L, Hartung HP, Weller M, et al. Interferon-beta enhances monocyte and dendritic cell expression of B7-H1 (PD-L1), a strong inhibitor of autologous

T-cell activation: Relevance for the immune modulatory effect in multiple sclerosis. J Neuroimmunol. 2004 Oct;155(1–2):172–82. http://dx.doi.org/10.1016/j.jneuroim.2004.06.013
47. Yearley JH, Gibson C, Yu N, Moon C, Murphy E, Juco J, et al. PD-L2 Expression in Human Tumors: Relevance to Anti-PD-1 Therapy in Cancer. Clinical cancer research. 2017 Jun 15;23(12):3158–67. PubMed PMID: 28619999.
48. Sheppard KA, Fitz LJ, Lee JM, Benander C, George JA, Wooters J, et al. PD-1 inhibits T-cell receptor induced phosphorylation of the ZAP70/CD3zeta signalosome and downstream signaling to PKCtheta. FEBS Lett. 2004 Sep 10;574(1–3):37–41. http://dx.doi.org/10.1016/j.febslet.2004.07.083
49. Yokosuka T, Takamatsu M, Kobayashi-Imanishi W, Hashimoto-Tane A, Azuma M, Saito T. Programmed cell death 1 forms negative costimulatory microclusters that directly inhibit T cell receptor signaling by recruiting phosphatase SHP2. J Exp Med. 2012 Jun 04;209(6):1201–17. http://dx.doi.org/10.1084/jem.20112741
50. Blank C, Brown I, Peterson AC, Spiotto M, Iwai Y, Honjo T, et al. PD-L1/B7H-1 inhibits the effector phase of tumor rejection by T cell receptor (TCR) transgenic CD8+ T cells. Cancer Res. 2004 Feb 01; 64(3):1140–5. http://dx.doi.org/10.1158/0008-5472.CAN-03-3259
51. Ahmadzadeh M, Johnson LA, Heemskerk B, Wunderlich JR, Dudley ME, White DE, et al. Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. Blood. 2009 Aug 20;114(8):1537–44. http://dx.doi.org/10.1182/blood-2008-12-195792
52. Baitsch L, Baumgaertner P, Devevre E, Raghav SK, Legat A, Barba L, et al. Exhaustion of tumor-specific CD8(+) T cells in metastases from melanoma patients. J Clin Invest. 2011 Jun;121(6):2350–60. http://dx.doi.org/10.1172/JCI46102
53. Chapon M, Randriamampita C, Maubec E, Badoual C, Fouquet S, Wang SF, et al. Progressive upregulation of PD-1 in primary and metastatic melanomas associated with blunted TCR signaling in infiltrating T lymphocytes. J Invest Dermatol. 2011 Jun;131(6):1300–7. http://dx.doi.org/10.1038/jid.2011.30
54. Gros A, Robbins PF, Yao X, Li YF, Turcotte S, Tran E, et al. PD-1 identifies the patient-specific CD8(+) tumor-reactive repertoire infiltrating human tumors. J Clin Invest. 2014 May;124(5):2246–59. http://dx.doi.org/10.1172/JCI73639
55. Inozume T, Hanada K, Wang QJ, Ahmadzadeh M, Wunderlich JR, Rosenberg SA, et al. Selection of CD8+PD-1+ lymphocytes in fresh human melanomas enriches for tumor-reactive T cells. J Immunother. 2010 Nov–Dec;33(9):956–64. http://dx.doi.org/10.1097/CJI.0b013e3181fad2b0
56. Taube JM, Anders RA, Young GD, Xu H, Sharma R, McMiller TL, et al. Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Sci Transl Med. 2012 Mar 28;4(127):127ra37. http://dx.doi.org/10.1126/scitranslmed.3003689
57. Noguchi T, Ward JP, Gubin MM, Arthur CD, Lee SH, Hundal J, et al. Temporally distinct PD-L1 expression by tumor and host cells contributes to immune escape. Cancer Immunol Res. 2017 Feb;5(2):106–17. http://dx.doi.org/10.1158/2326-6066.CIR-16-0391
58. Taube JM, Klein A, Brahmer JR, Xu H, Pan X, Kim JH, et al. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. Clin Cancer Res. 2014 Oct 01;20(19):5064–74.
59. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014 Nov 27;515(7528):568–71. http://dx.doi.org/10.1038/nature13954
60. Pauken KE, Sammons MA, Odorizzi PM, Manne S, Godec J, Khan O, et al. Epigenetic stability of exhausted T cells limits durability of reinvigoration by PD-1 blockade. Science. 2016 Dec 02;354(6316):1160–5. http://dx.doi.org/10.1126/science.aaf2807
61. Kleffel S, Posch C, Barthel SR, Mueller H, Schlapbach C, Guenova E, et al. Melanoma cell-intrinsic PD-1 receptor functions promote tumor growth. Cell. 2015 Sep 10;162(6):1242–56. http://dx.doi.org/10.1016/j.cell.2015.08.052
62. Weber JS, D'Angelo SP, Minor D, Hodi FS, Gutzmer R, Neyns B, et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): A randomised, controlled, open-label, phase 3 trial. Lancet Oncol. 2015 Apr;16(4):375–84. http://dx.doi.org/10.1016/S1470-2045(15)70076-8

63. Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med. 2015 Jan 22;372(4):320–30. http://dx.doi.org/10.1056/NEJMoa1412082
64. Larkin J, Hodi FS, Wolchok JD. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med. 2015 Sep 24;373(13):1270–1. http://dx.doi.org/10.1056/NEJMc1509660
65. Ribas A, Puzanov I, Dummer R, Schadendorf D, Hamid O, Robert C, et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): A randomised, controlled, phase 2 trial. Lancet Oncol. 2015 Aug;16(8):908–18. http://dx.doi.org/10.1016/S1470-2045(15)00083-2
66. Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, et al. Pembrolizumab versus ipilimumab in advanced melanoma. N Engl J Med. 2015 Jun 25;372(26):2521–32. http://dx.doi.org/10.1056/NEJMoa1503093
67. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. Science. 2015 Apr 03;348(6230):69–74. http://dx.doi.org/10.1126/science.aaa4971
68. van Rooij N, van Buuren MM, Philips D, Velds A, Toebes M, Heemskerk B, et al. Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma. J Clin Oncol. 2013 Nov 10;31(32):e439–42. http://dx.doi.org/10.1200/JCO.2012.47.7521
69. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med. 2014 Dec 4;371(23):2189–99. http://dx.doi.org/10.1056/NEJMoa1406498
70. Van Allen EM, Miao D, Schilling B, Shukla SA, Blank C, Zimmer L, et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. Science. 2015 Oct 09;350(6257):207–11. http://dx.doi.org/10.1126/science.aad0095
71. McGranahan N, Furness AJ, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science. 2016 Mar 25;351(6280):1463–9. http://dx.doi.org/10.1126/science.aaf1490
72. Ribas A, Shin DS, Zaretsky J, Frederiksen J, Cornish A, Avramis E, et al. PD-1 blockade expands intratumoral memory T cells. Cancer Immunol Res. 2016 Mar;4(3):194–203. http://dx.doi.org/10.1158/2326-6066.CIR-15-0210
73. Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014 Nov 27;515(7528):563–7. http://dx.doi.org/10.1038/nature14011
74. Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, et al. Commensal bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Science. 2015 Nov 27;350(6264):1084–9. http://dx.doi.org/10.1126/science.aac4255
75. Vetizou M, Pitt JM, Daillere R, Lepage P, Waldschmitt N, Flament C, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science. 2015 Nov 27;350(6264):1079–84. http://dx.doi.org/10.1126/science.aad1329
76. Huang AC, Postow MA, Orlowski RJ, Mick R, Bengsch B, Manne S, et al. T-cell invigoration to tumour burden ratio associated with anti-PD-1 response. Nature. 2017 May 4;545(7652):60–5. http://dx.doi.org/10.1038/nature22079
77. Blank CU, Haanen JB, Ribas A, Schumacher TN. CANCER IMMUNOLOGY. The "cancer immunogram." Science. 2016 May 06;352(6286):658–60. http://dx.doi.org/10.1126/science.aaf2834
78. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: Integrating immunity's roles in cancer suppression and promotion. Science. 2011 Mar 25;331(6024):1565–70. http://dx.doi.org/10.1126/science.1203486
79. Sucker A, Zhao F, Real B, Heeke C, Bielefeld N, Mabetaen S, et al. Genetic evolution of T-cell resistance in the course of melanoma progression. Clin Cancer Res. 2014 Dec 15;20(24):6593–604.
80. Anagnostou V, Smith KN, Forde PM, Niknafs N, Bhattacharya R, White J, et al. Evolution of neoantigen landscape during immune checkpoint blockade in non-small cell lung cancer. Cancer Discov. 2017 Mar;7(3):264–76. http://dx.doi.org/10.1158/2159-8290.CD-16-0828
81. Zaretsky JM, Garcia-Diaz A, Shin DS, Escuin-Ordinas H, Hugo W, Hu-Lieskovan S, et al. Mutations associated with acquired resistance to PD-1 blockade in melanoma. N Engl J Med. 2016 Sep 01;375(9):819–29. http://dx.doi.org/10.1056/NEJMoa1604958

82. Liu C, Peng W, Xu C, Lou Y, Zhang M, Wargo JA, et al. BRAF inhibition increases tumor infiltration by T cells and enhances the antitumor activity of adoptive immunotherapy in mice. Clin Cancer Res. 2013 Jan 15;19(2):393–403.
83. Peng W, Chen JQ, Liu C, Malu S, Creasy C, Tetzlaff MT, et al. Loss of PTEN promotes resistance to T cell-mediated immunotherapy. Cancer Discov. 2016 Feb;6(2):202–16. http://dx.doi.org/10.1158/2159-8290.CD-15-0283
84. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. Nature. 2015 Jul 09;523(7559):231–5. http://dx.doi.org/10.1038/nature14404
85. Platanias LC. Mechanisms of type-I- and type-II-interferon-mediated signalling. Nat Rev Immunol. 2005 May;5(5):375–86. http://dx.doi.org/10.1038/nri1604
86. Gao J, Shi LZ, Zhao H, Chen J, Xiong L, He Q, et al. Loss of IFN-gamma pathway genes in tumor cells as a mechanism of resistance to anti-CTLA-4 therapy. Cell. 2016 Oct 06;167(2):397–404 e9.
87. Lee JH, Wang LC, Lin YT, Yang YH, Lin DT, Chiang BL. Inverse correlation between CD4+ regulatory T-cell population and autoantibody levels in paediatric patients with systemic lupus erythematosus. Immunology. 2006 Feb;117(2):280–6. http://dx.doi.org/10.1111/j.1365-2567.2005.02306.x
88. Shin DS, Zaretsky JM, Escuin-Ordinas H, Garcia-Diaz A, Hu-Lieskovan S, Kalbasi A, et al. Primary resistance to PD-1 blockade mediated by JAK1/2 mutations. Cancer Discov. 2017 Feb;7(2):188–201. http://dx.doi.org/10.1158/2159-8290.CD-16-1223
89. Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: A common denominator approach to cancer therapy. Cancer Cell. 2015 Apr 13;27(4):450–61. http://dx.doi.org/10.1016/j.ccell.2015.03.001
90. Lin RL, Zhao LJ. Mechanistic basis and clinical relevance of the role of transforming growth factor-beta in cancer. Cancer Biol Med. 2015 Dec;12(4):385–93.
91. Beavis PA, Milenkovski N, Henderson MA, John LB, Allard B, Loi S, et al. Adenosine Receptor 2A blockade increases the efficacy of anti-PD-1 through enhanced antitumor T-cell responses. Cancer Immunol Res. 2015 May;3(5):506–17. http://dx.doi.org/10.1158/2326-6066.CIR-14-0211
92. Talmadge JE, Gabrilovich DI. History of myeloid-derived suppressor cells. Nat Rev Cancer. 2013 Oct;13(10):739–52. http://dx.doi.org/10.1038/nrc3581
93. Yang L, DeBusk LM, Fukuda K, Fingleton B, Green-Jarvis B, Shyr Y, et al. Expansion of myeloid immune suppressor Gr+CD11b+ cells in tumor-bearing host directly promotes tumor angiogenesis. Cancer Cell. 2004 Oct;6(4):409–21. http://dx.doi.org/10.1016/j.ccr.2004.08.031
94. Yang L, Huang J, Ren X, Gorska AE, Chytil A, Aakre M, et al. Abrogation of TGF beta signaling in mammary carcinomas recruits Gr-1+CD11b+ myeloid cells that promote metastasis. Cancer Cell. 2008 Jan;13(1):23–35. http://dx.doi.org/10.1016/j.ccr.2007.12.004
95. Meyer C, Cagnon L, Costa-Nunes CM, Baumgaertner P, Montandon N, Leyvraz L, et al. Frequencies of circulating MDSC correlate with clinical outcome of melanoma patients treated with ipilimumab. Cancer Immunol Immunother. 2014 Mar;63(3):247–57.
96. Weber J, Gibney G, Kudchadkar R, Yu B, Cheng P, Martinez AJ, et al. Phase I/II study of metastatic melanoma patients treated with nivolumab who had progressed after ipilimumab. Cancer Immunol Res. 2016 Apr;4(4):345–53. http://dx.doi.org/10.1158/2326-6066.CIR-15-0193
97. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. Cell. 2017 Feb 09;168(4):707–23. http://dx.doi.org/10.1016/j.cell.2017.01.017
98. Boni A, Cogdill AP, Dang P, Udayakumar D, Njauw CN, Sloss CM, et al. Selective BRAFV600E inhibition enhances T-cell recognition of melanoma without affecting lymphocyte function. Cancer Res. 2010 Jul 01;70(13):5213–19. http://dx.doi.org/10.1158/0008-5472.CAN-10-0118
99. Bradley SD, Chen Z, Melendez B, Talukder A, Khalili JS, Rodriguez-Cruz T, et al. BRAFV600E co-opts a conserved MHC class I internalization pathway to diminish antigen presentation and CD8+ T-cell recognition of melanoma. Cancer Immunol Res. 2015 Jun;3(6):602–9. http://dx.doi.org/10.1158/2326-6066.CIR-15-0030
100. Frederick DT, Piris A, Cogdill AP, Cooper ZA, Lezcano C, Ferrone CR, et al. BRAF inhibition is associated with enhanced melanoma antigen expression and a more favorable tumor microenvironment in patients with metastatic melanoma. Clin Cancer Res. 2013 Mar 01;19(5):1225–31.
101. Wilmott JS, Long GV, Howle JR, Haydu LE, Sharma RN, Thompson JF, et al. Selective BRAF inhibitors induce marked T-cell infiltration into human metastatic melanoma. Clin Cancer Res. 2012 Mar 01;18(5):1386–94.

102. Sapkota B, Hill CE, Pollack BP. Vemurafenib enhances MHC induction in BRAFV600E homozygous melanoma cells. Oncoimmunology. 2013 Jan 01;2(1):e22890. http://dx.doi.org/10.4161/onci.22890
103. Ebert PJ, Cheung J, Yang Y, McNamara E, Hong R, Moskalenko M, et al. MAP kinase inhibition promotes T cell and anti-tumor activity in combination with PD-L1 checkpoint blockade. Immunity. 2016 Mar 15;44(3):609–21. http://dx.doi.org/10.1016/j.immuni.2016.01.024
104. Kaneda MM, Messer KS, Ralainirina N, Li H, Leem CJ, Gorjestani S, et al. PI3Kgamma is a molecular switch that controls immune suppression. Nature. 2016 Nov 17;539(7629):437–42. http://dx.doi.org/10.1038/nature19834
105. De Henau O, Rausch M, Winkler D, Campesato LF, Liu C, Cymerman DH, et al. Overcoming resistance to checkpoint blockade therapy by targeting PI3Kgamma in myeloid cells. Nature. 2016 Nov 17;539(7629):443–7. http://dx.doi.org/10.1038/nature20554

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Nanomedicine in Melanoma: Current Trends and Future Perspectives

AYMAN EL-MEGHAWRY EL-KENAWY[1,2*] • CAROLINA CONSTANTIN[3,4] • SNUR M. A. HASSAN[5] • ALSHIMAA MOHAMED MOSTAFA[6] • ADRIANA FREITAS NEVES[7] • THAISE GONÇALVES DE ARAÚJO[8] • MONICA NEAGU[3,4,9]

[1]Department of Molecular Biology, Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Sadat, Egypt; [2]Department of Pathology, College of Medicine, Taif University, Taif, Saudi Arabia; [3]Immunology Department, Victor Babes National Institute of Pathology, Bucharest, Romania; [4]Colentina University Hospital, Bucharest, Romania; [5]Department of Anatomy and Pathology, College of Veterinary Medicine, Sulaimani University, Sulaymaniyah, Kurdistan-Iraq; [6]Department of Dermatology, Faculty of Medicine, Beni-Suef University, Beni-Suef, Egypt; [7]Molecular Biology Laboratory, Institute of Biotechnology, Universidade Federal de Goias, Catalao, Brazil; [8]Laboratory of Genetics and Biotechnology, Institute of Genetics and Biochemistry, Federal University of Uberlandia, Patos de Minas, Brazil; [9] Faculty of Biology, University of Bucharest, Bucharest, Romania

Author for correspondence: Ayman El-Meghawry El-Kenawy, Department of Pathology, College of Medicine, Taif University, Saudi Arabia. Email: elkenawyay@yahoo.com

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Abstract: As cutaneous melanoma is a highly aggressive and drug-resistant cancer, there is intense research focusing on developing new, efficient drugs. Nanomedicine focuses on developing different groups of nanomaterials for both diagnosis and therapy, and this combination of specific diagnosis and therapy is called theranostics. Nanomaterials tailored as delivery vehicles can be nanocapsules, nanorods,

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nanotubes, nanoshells, and nanocages. All these structures protect the intended drug against degradation and enhance its stability. The development and characterization of polymeric nanoparticles, polymeric micelles, liposomes, nanohydrogel, dendrimers, inorganic nanoparticles, and hybrid nanocarriers are among the delivery vehicles that transport different anticancer agents. Functionalization of nanocarriers with specific molecules, such as antibodies, can generate different smart nanodrugs for application in cancer therapy and/or diagnosis. Nanotherapeutic strategies deal with several shortcomings that comprise of tumor characteristics, biological barriers, biocompatibility, and so on. As nanostructures interact with various host biomolecules, comprehensive *in vitro* cellular models call for evaluation of physicochemical properties, dose, and time of action of nanomaterials, while *in vivo* assessments would provide valuable data regarding the level of absorption, tissue/organ distribution, and metabolism. The future perspectives in nanotechnology applied to cancer overcomes the translational barrier from the laboratory to the clinical application to potentially improve conventional theranostic techniques.

Key words: Melanoma; Nanomedicine; Nanotechnology; Theranostic; Treatment

Introduction

Melanoma, the cancer of melanocytes, is the sixth most frequently diagnosed cancer in humans and accounts for 80% of skin cancer–related deaths (1). Morbidity and mortality indices are highly variable worldwide—being rare in nations of Asian and African origin and almost considered epidemic in countries of Caucasian predominance (2, 3). When diagnosed early, as a localized cutaneous tumor, melanoma can be surgically removed with a good prognosis (4). Once melanoma becomes metastatic, it turns into a more aggressive and difficult to treat malignancy (5). Management of metastatic melanoma is challenging if the tumor becomes unresectable or if it recurs shortly after resection (6). In such cases, other conventional treatment options including chemotherapy, radiotherapy, targeted therapy, and photodynamic and immunotherapy have to be combined with surgery (7).

Dacarbazine (DTIC) is the first chemotherapeutic treatment approved by U.S. Food and Drug Administration (FDA) for metastatic melanoma (8). Temozolomide, a DTIC derivative, has the ability to cross the blood–brain barrier and is a first-line therapy for brain metastases (9). Recently, BRAF inhibitors (Vemurafenib, Dabrafenib) and MEK inhibitor (Trametinib) have been approved by the FDA for treating BRAF-mutated melanoma which is nearly found in 50% of cases (2). Immunotherapy is another promising treatment option in metastatic melanoma. Ipilimumab, an anti-cytotoxic T-lymphocyte antigen 4 antibody (CTLA-4), and nivolumab and pembrolizumab, programmed death receptor 1 (PD-1) inhibitors, have been approved for use in the treatment of metastatic melanoma (10, 11). However, despite these recent therapeutic breakthroughs, there are still some drawbacks including undesirable side effects, tumor chemoresistance, or even disease relapse (2, 12). As cutaneous melanoma is a highly aggressive cancer (13), there is intense research focus on developing new, efficient drugs. Taken together,

these challenges have led researchers to explore new ways of early diagnosis and investigate novel approaches of drug delivery to reach high efficacy, minimal toxicity, and less failure—advantages that melanoma-related nanotechnology could potentially offer (14, 15).

Nanomedicine for Melanoma Detection and Treatment

Early diagnosis of melanoma is essential to increase patients' survival rates. The 10-year survival rate for Stage IA is 93%, while patients diagnosed at Stage IV have a 10-year survival rate of 10–15% only (16–18). Moreover, the cost of treating melanoma increases dramatically with later stages of the disease (19–21). In addition to the clinical and histological examination, many new techniques have been utilized to aid early detection of melanoma. These techniques include dermoscopy, total body photography, multispectral digital imaging analysis, and RNA microarray (22, 23). In-depth investigations of the molecular changes of metastatic melanoma have paved the way for more advanced technologies known as molecular diagnostics. They include fluorescent *in situ* hybridization (FISH), next-generation sequencing (NGS), quantitative reverse transcription-polymerase chain reaction (qRT-PCR), comparative genome hybridization (CGH), and detection of exosomes (24–27). Nanotechnology is one of the promising tools recently used for detection of melanoma with high sensitivity and specificity (28–30).

Nanoparticle quantum dots (QDs), fluorescent nanoparticles characterized by excellent brightness, narrow field of emissions, broad absorption spectrum, and excellent photostability, have been suggested as a useful technique for cancer detection (31–35). Those photophysical properties allowed researchers to conjugate QDs with variable cancer-specific molecules as folic acid or antibodies against specific cancer antigens (36–40). When QDs are conjugated with specific anti-melanoma antibodies (e.g., HMB45, MART-1, and Tyrosinase), melanoma cells can be distinguished from normal melanocytes (41). However, the heavy metal composition of QDs, with its high toxicity and immunogenicity, hinders the wide application of QDs as an imaging modality for cancer (42, 43). Recently, coating QDs with a polyethylene glycol (PEG) have been shown to decrease cytotoxicity (44). Similarly, Cornell dots known as C-Dots are PEG-coated silica-based nanoparticles that are used as probes to guide sentinel lymph node biopsy (SLNB) (45, 46). These FDA-approved nanoparticles are used as PET-optical or optical probes that particularly target RGD peptides attached to alpha 2 beta 3 integrin overexpressed in melanoma cells (47–50). Nanotechnology has been used in medicine for developing nanometer scale materials, ranging from 1 to 100 nm, having therapeutic and diagnostic purposes (51–53). Nanomaterials' size range matches cellular organelles, other molecules involved in intracellular events, as signaling pathways, and/or molecules involved in cell to cell communication (16, 20). The nanomaterials bio-distribution is dependent on the surface charge, biodegradability, size, their distinct biological properties, and shape (19, 21–25). Nanoparticles (NPs) or nanocapsules are the most common shape for nanomaterials used as drug delivery systems. Moreover, this shape offers protection against degradation, enhances its stability, driving an efficient accumulation at target sites (26).

Currently, nanorods, nanotubes, nanoshells, and nanocages are nanomaterials with imaging and cancer therapy applications (26, 27).

Carbon-based nanoparticles are effective in melanoma cells (53). Thus, a single-walled carbon nanotube loaded with DOX-induced melanoma cell death in a dose-dependent manner *in vitro* and revoked tumor development in a xenograft melanoma model. Gold nanoparticles (GNPs) are known as nontoxic, highly stable, easy to synthesize, and minimally interfering with the biological profile of melanoma tumor cell (54, 55). Being of high atomic number and electron density, GNPs are optimal contrast agents for computed tomography (CT) (56, 57). When labeled with radioisotope indium-111 and conjugated with RGD ligands, GNPs were successfully used as radiotracers in experimental melanoma models (58). Meir et al. have shown in melanoma-bearing mice that labeled GNPs can track tumor-specific T-cells using whole body CT. This approach is a next-generation imaging technique as well as a new tool in immunotherapy (59).

Magnetic nanoparticles (MNPs) were successfully used in MRI (60). In the recent MELAMAG clinical trial, SLNB detection based on MNPs was compared to the standard technique. In this study, MNPs with small iron nanoparticles (named Sienna+ by the developers) were intradermally injected and a hand-held magnetometer was used intraoperatively to detect the accumulation of MNPs. A gamma probe was used as comparator and the results showed the feasibility of the magnetic technique for SLNB detection. The highest identification was proven for inguinal and axillary lymph nodes, while the lowest detection rates were registered for the cervical region. From 129 recruited patients, the study reported 95.3% rate of sentinel node identification using this MNPs-based technique (60).

Another nanoparticle tested for contrast-enhanced MRI lymphography is Gadolinium-loaded nanoparticles (Gd-FVT). Using these NPs, the specificity and sensitivity of MRI lymphography in melanoma-bearing mice could be enhanced (61).

Zhou et al. developed an efficient and noninvasive strategy to detect melanoma metastasis in LN using Gd-embedded iron oxide nanoplates (GdIOP), functionalized with Zwitterionic Dopamine Sulfonate (ZDS) molecules. With T1-T2 dual-modal MRI, GdIOP@ZDS nanoparticles were highly taken up by dendritic cells and macrophages in LN, in contrast to melanoma B16 tumor cells which showed lower uptake. This generated difference represented pseudo-contrast images which can be potentially used for detection of melanoma metastasis in LN (62).

RGD-targeted nanoparticles of iron oxide (NPIO) were previously utilized for MRI of *in vivo* tumor angiogenesis with variable limitations including long blood half-life and nonspecific extravasation (63). Nevertheless, conjugation of cyclic RGD variant [c (RGDyK)], with enhanced affinity for $\alpha v\beta 3$, a specific marker of angiogenesis, to iron oxide microparticles (MPIO) provided a more sensitive molecular MRI approach (64).

Another promising application of nanotechnology is the detection and quantification of circulating tumor cells (CTCs) as a blood-based biomarker "liquid biopsy" (65). Seenivasan et al. immobilized anti-Melanocortin 1 receptor antibodies (MC1R-Abs) on amino-functionalized silica nanoparticles (n-SiNPs)-polypyrrole (PPy) nanocomposite thin film and used them as an immune sensor

for selective and sensitive detection of melanoma cells (66). A magnetite nanoparticle designed by Sato et al. by conjugating N-propionyl-cyst aminyl phenol with magnetite was used in a B16F1 xenograft mouse model (67). Souza et al. showed that melanoma cells were degraded after the application of an external irregular magnetic field to increase the temperature in the tumor to 43°C. The nanoparticle had a 1.7- to 5.4-fold greater effect compared with magnetite alone (46).

Nano Therapies: Radiotherapy and Chemotherapy

NPs in the context of radiotherapy and chemotherapy are particularly interesting. Radiotherapy and surgery are local treatments, while the main systemic strategy is chemotherapy, especially considering the risk of metastasis (30). Radiotherapy has a limited role in treating melanoma patients and is used selectively. Its success is limited due to radiation resistance in melanoma cells (16, 34). This technique has been improved by engineering, physics, chemistry, and biology to promote innovative technologies that allow real-time imaging and better dose distribution according to disease progress (67).

In general, the radioisotopes used in medicine emit energy that produces DNA cleavage, damage that is induced mainly by ionized atoms and free radicals. The clearance performed by the kidney is dependent on the size of the radioisotopes. Molecules smaller than 5 nm are excreted rapidly and fail in promoting desirable effects due to short circulation time in blood. Immune response, including opsonization, is another way for radioisotopes clearance by mononuclear phagocytes (MPS). In this context, nanocarriers emerge as an alternative for the half-life increment of radioisotopes (67). Glutathione-coated gold nanostructures represents the next generation of radiosensitizers used for gamma-ray irradiation (34, 68). Moreover, PEGylation of NPs produces nanocarriers that prevent opsonization, increasing the half-life of the radioisotopes (69). Carbon nanostructures have also been related as potential nanocarriers used in radiotherapy, displaying particular physicochemical properties (70) as ultralight, conductivity, and high-surface area (71).

POLYMERIC NANOPARTICLES

Polymeric nanoparticles (PNs) are molecules usually organized with tunable size into a dense structure with entangling biodegradable polymers presenting thermodynamic stability in an aqueous solvent (72–75). Recently, FDA approved three PNs, namely, polylactic acid (PLA), poly (lactic-co-glycolic acid) 43 (PLGA), and polycaprolactone (PCL). The hydrophilicity for the encapsulation of hydrophilic drugs is one of the deficiencies for the desired release of the encapsulated agents (32). Copolymers as polyethylene glycol (PEG)ylated have been used to reduce the degradation rate of PN to produce PLA-PEG, PLGA-PEG, improving their biocompatibility and modifying its amphiphilicity. Furthermore, PEG has been described as a strategy to evade immune response (76, 77).

LIPOSOMES AND NIOSOMES

Liposomes can remain in the blood circulation longer, permitting continued drug release with increased precision in tumor-targeting (78–84). They can incorporate nucleic acids and other organic or inorganic molecules into their aqueous lumen (85–89) and can be used for targeted, controlled drug release (90–96).

Thus, in two melanoma xenograft models, phosphatidylethanolamine liposomal cisplatin was proven to have higher cytotoxicity than classic liposomes or free cisplatin, a high concentration of intratumoral drug remaining for 72 h and efficiently delivering 3.6-times more drug compared to the free drug (97–103). Niosomes are biodegradable, biocompatible, nontoxic, and nonimmunogenic having extensive solubility and flexibility. Niosomes have been confirmed to have prolonged circulation, increased drug retention in skin, and enhanced drug spreading when topically applied (104–107). Dwivedi et al. proved that encapsulated artemisone which is a 10-amino-artemisinin derivative with antitumor activity in niosomes exhibited extremely selective cytotoxicity toward the melanoma cells but not to the normal skin cells (108).

NANOHYDROGELS

Nanohydrogels are cross-linked hydrophilic soft polymers organized in a tridimensional network comprising a large fraction of water (28, 32). The nanohydrogels' cross-linking occurs through hydrophilic–hydrophobic interactions, hydrogen bonds, electrostatic interactions, or covalent bonds. The aqueous environment promotes the swelling of nanohydrogels, a characteristic that is determined by the degree of the cross-linking and external environment. This nanocarrier is promising for multimodality treatment, especially for peptides, proteins, and oligonucleotides, because of their hydrophilicity and efficient cell uptake. The co-delivery of PTX and DOX drugs in nanogels are possible due to the positively charged surface that could load negatively charged proteins (32). Functionalized nanohydrogels siRNA delivery systems that target epidermal growth factor receptor were tested in an ovarian cancer mouse model in a platinum-based therapy (82). Polymersome could be valuable for melanoma treatment owing to its benefits, such as robustness, increased drug loading, constancy, relatively longer *in vivo* circulation, and the possibility to design it for the delivery of multiple drugs (104). Polymersomes have been used to carry DOX for melanoma therapy and established to be specially taken up by melanoma cells (109–111).

THERANOSTIC NANOMEDICINE AND MELANOMA THERAPY

By using nanoparticles for both diagnosis and treatment, theranostic nanomedicine has been advanced recently (112, 113). Liposomes, exosomes, polymersomes, nanocrystals, nanotubes, and nanowires are among the commonly used nanoparticles and nanodevices, and endless combinations can be created with these nanostructures (114). Some metals, such as gold (Au) and Gadolinium (Gd), can have antitumor activity besides being an imaging tracer (88). Gd-based NPs (AGuIX) were successfully used as both MRI contrast agent and therapy in

experimental animal models of melanoma metastases (115–120). Another novel theranostic nanostructure for melanoma was a NP biodegradable photoluminescent polymer—poly (lactic acid) (BPLP-PLA) loaded with anti-BRAF V600E–specific drug (PLX4032) and muramyl peptide. The new immune-cell-mediated nanoparticle offers high hopes for melanoma imaging and treatment (121–126).

Current Limitations and Exploring Possibilities for Improving the Efficiency of Nanodrugs in Melanoma

Although notable progress has been made in the synthesis and characterization of nanodrugs, and we are witnessing the first clinical trials that have shown promise (127–130), there are still limitations that should be overcome. Thus, nanodrugs, once having entered the biological system, complexly interact with the host's immune system, leading to premature clearance, side-effect activation, and toxicity (131–135). Consequently, the main limitations of nanodrug efficacy are the immunological interactions, the biological barriers that hinder the availability of nanodrugs to the intended target, and the heterogeneity of the biological target (38, 136).

In order to improve their efficacy, nanodrugs should overcome these major limitations and several means of overcoming them are further described in this subsection. An overview of the main issues discussed in this chapter is presented in Figure 1.

NANODRUGS' ACTION IS LIMITED BY THE INTERACTION WITH THE BIOLOGICAL SYSTEM

There are complex interactions of nanodrugs once introduced in a biological system, because they would interact with cellular and humoral constituents of the immune system. Thus, the transition to routine clinical application of these

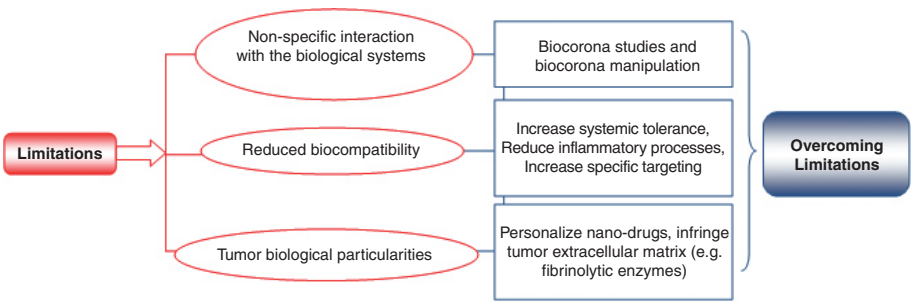

Figure 1 Main limitations of nanodrug efficiency in antitumoral therapy and possibilities to overcome the limitations.

nanocompounds would be hampered first by different biological barriers and second by their uncertain fate at the diseased site (136, 137).

In the biological system, nanomaterials interact with all the encountered biomolecules and dynamically form the so called "bio-corona." The commonly agreed definition of the bio-corona is the multitude and the variety of biomolecules (e.g., proteins, peptides, and lipids) that associate with the surface of a nanoparticle when introduced in a biological system. The process of entrapping nanoparticles within complex surface biomolecules bequeaths them with properties that can hinder the actual intended properties of the nanodrug. Undeniably, the bio-corona establishment controls the nanodrug efficacy and further focuses the actions of natural and adaptive immunity (138). It is not surprising that nanomaterials are directly interacting with the immune system. The evolvement of human immune system was accomplished through exposure to different chemical, physical, and biological agents (139). As NPs are in the size range of biological aggressors, interactions with the immune system are more likely to occur. Thus, a major limitation in nanomedicine is the correct evaluation of the fate of the nanodrugs as antitumoral effectors within a biological system (140–146). As nanomaterials match the same size range as biomolecules and cellular structures endows them with the propensity to reach intracellular structures previously accessible only to biological aggressors. Alternatively, as they have already encountered the complex biological milieu and interacted with other biomolecules, they are subjected to intracellular pathways that are not the intended targets. Hence *in vitro* investigation of the bio-corona dynamics should be performed to be assured that within the biological system the nanodrugs will reach their intended cellular target (129).

Taking advantage of phagocytes' capacity to engulf NPs, recently, a novel drug delivery system was reported using macrophages as both carriers and effector cells upon melanoma cells. Hence, nanoparticles of biodegradable photoluminescent poly (lactic acid) were loaded with a drug specific for anti-BRAF V600E mutant melanoma forming a complex engulfed further by macrophages which would directly bind and kill melanoma tumor cells (147–150).

Conclusion

Novel treatment methods should have several properties. For example, they should be more effective, cheaper, and without any risk to patient life, even if they do not improve patient's quality of life. To accomplish patient safety, and for good patient compliance, an ideal treatment should be developed with an improved overall treatment efficiency, a very low possible toxicity, and a specific targeted site (91). Nanotechnology-based formulations can provide all of the above, and their efficacy can be further improved when ornamented with targeting moieties, for instance, specific antibodies (92) or targeted delivery payload (93–95). In the last 5 years, there has been an exponential increase in the focus on nanotechnology with regard to melanoma therapy and related diagnosis (96). Nanomedicine is a new area that develops nanotechnology for therapeutic and diagnostic purposes. Nowadays, different groups of nanomaterials have been designed for drug delivery and/or for identifying specific markers.

Nanomaterials as delivery vehicles can be nanocapsules, nanorods, nanotubes, nanoshells, and nanocages—structures that protect the drug against degradation, thereby enhancing its stability. The development and characterization of PNs, polymeric micelles, liposomes, nanohydrogel, dendrimers, inorganic nanoparticles, and hybrid nanocarriers are among the delivery vehicles that transport different anticancer agents. Chemical drugs, nucleic acids, proteins, antibodies, and functionalization of nanocarriers with inorganic compounds such as magnetic, graphene oxide, carbon, silica, gold and QDs in a core-shell system can generate smart nanodrugs for application in cancer therapy and/or diagnosis. In therapy for skin melanoma, as well as for other tumors, nanotherapeutic strategies deal with several shortcomings that comprise of tumor characteristics, biological barriers, and biocompatibility. Toxicological profile of nanoparticles should be robustly assessed. When systemically administered, nanostructures interact with various host biomolecules, and may trigger toxicity (151, 152). Therefore, comprehensive *in vitro* cellular models call for evaluation of physicochemical properties, dose, and time of action of nanomaterials, while *in vivo* assessments would provide valuable data regarding level of absorption, tissue/organ distribution, and metabolism (153). Although preclinical investigations are essential for assessing the potential health risks of nanostructures, animal models retain significant limitations and the human system may react differently to a certain drug compared to animal models (154). Last but not least, the translation of nanodrugs from preclinical to clinical stage is a major issue still unsettled in melanoma nanomedicine area. The future perspectives in nanotechnology applied to cancer is very promising in improving cancer management.

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References

1. Bertolotto C. Melanoma: From melanocyte to genetic alterations and clinical options. Scientifica. 2013;12:1–22. http://dx.doi.org/10.1155/2013/635203
2. Bombelli FB, Webster CA, Moncrieff M, Sherwood V. The scope of nanoparticle therapies for future metastatic melanoma treatment. Lancet Oncol. 2014;15(1):22–32.

3. Heo JR, Kim NH, Cho J, Choi KC. Current treatments for advanced melanoma and introduction of a promising novel gene therapy for melanoma (Review). Onco Rep. 2016;36(4):1779–86.
4. Maverakis E, Cornelius LA, Bowen GM, Phan T, Patel FB, Fitzmaurice S, et al. Metastatic melanoma—A review of current and future treatment options. Acta Dermato Venereolog. 2015;95(5):516–27.
5. Younes R, Abrao FC, Gross J. Pulmonary metastasectomy for malignant melanoma: Prognostic factors for long-term survival. Melanoma Res. 2013;23:307–11. http://dx.doi.org/10.1097/CMR.0b013e3283632cbe
6. Brys AK, Gowda R, Loriaux DB, Robertson GP, Mosca PJ. Nanotechnology-based strategies for combating toxicity and resistance in melanoma therapy. Biotech Adv. 2016;34(5):565–77.
7. Lloyd-Hughes H, Shiatis AE, Pabari A, Mosahebi A, Seifalian A. Current and future nanotechnology applications in the management of melanoma: A review. J Nanomed Nanotechnol 2015;6:334. http://dx.doi.org/10.4172/2157-7439.1000334
8. Kim T, Amaria RN, Spencer C, Reuben A, Cooper ZA, Wargo JA. Combining targeted therapy and immune checkpoint inhibitors in the treatment of metastatic melanoma. Cancer Biol Med. 2014;11:237–46.
9. Schindler AK, Postow MA. Current options and future directions in the systemic treatment of metastatic melanoma. J Com Sup Oncol. 2014;12:20–6. http://dx.doi.org/10.12788/jcso.0005
10. Johnson DB, Peng C, Sosman JA. Nivolumab in melanoma: Latest evidence and clinical potential. Therap Adv Med Oncol. 2015;7:97–106. http://dx.doi.org/10.1177/1758834014567469
11. Long GV, Atkinson V, Ascierto PA, Dutriaux C, Maio M, Mortier L, et al. Nivolumab improved survival vs dacarbazine in patients with untreated advanced melanoma. J Transl Med. 2015;2:13. http://dx.doi.org/10.1186/1479-5876-13-S1-O6
12. Mundra V, Li W, Mahato RI. Nanoparticle-mediated drug delivery for treating melanoma. Nanomed. 2015;10(16):2613–33.
13. Ancuceanu R, Neagu M. Immune based therapy for melanoma. Indian J Med Res. 2016; 143(2):135–44. http://dx.doi.org/10.4103/0971-5916.180197
14. Chen J, Shao R, Zhang XD, Chen C. Applications of nanotechnology for melanoma treatment, diagnosis, and theranostics. Intl J Nanomed. 2013:8 2677–88. http://dx.doi.org/10.2147/IJN.S45429
15. Guy GP, Ekwueme DU, Tangka FK, Richardson LC. Melanoma treatment costs: A systematic review of the literature, 1990–2011. Am J Prev Med. 2012;43(5):537–45.
16. Akhtar MJ, Ahamed M, Alhadlaq HA. Therapeutic targets in the selective killing of cancer cells by nanomaterials. Clin Chim Acta Int J Clin Chem. 2017;469:53–62.
17. Doane TL, Burda C. The unique role of nanoparticles in nanomedicine: Imaging, drug delivery and therapy. Chem Soc Rev. 2012;41(7):2885–911. http://dx.doi.org/10.1039/c2cs15260f
18. Kim BY, Rutka JT, Chan WC. Nanomedicine. N Eng J Med. 2010;363(25):2434–43. http://dx.doi.org/10.1056/NEJMra0912273
19. Kumari B, Hora A, Mallik M. Nanomedicines in cancer research: An overview. LS Int J Life Sci. 2017;6:7. http://dx.doi.org/10.5958/2319-1198.2017.00002.1
20. Scheinberg DA, Grimm J, Heller DA, Stater EP, Bradbury M, McDevitt MR. Advances in the clinical translation of nanotechnology. Cur Opin Biotechnol. 2017;46:66–73. http://dx.doi.org/10.1016/j.copbio.2017.01.002
21. Adiseshaiah PP, Crist RM, Hook SS, McNeil SE. Nanomedicine strategies to overcome the pathophysiological barriers of pancreatic cancer. Nat Rev Clin Onco. 2016;13(12):750–65. http://dx.doi.org/10.1038/nrclinonc.2016.119
22. Fernandez-Fernandez A, Manchanda R, McGoron AJ. Theranostic applications of nanomaterials in cancer: Drug delivery, image-guided therapy, and multifunctional platforms. App Biochem Biotechnol. 2011;165(7–8):1628–51. http://dx.doi.org/10.1007/s12010-011-9383-z
23. Janib SM, Moses AS, MacKay JA. Imaging and drug delivery using theranostic nanoparticles. Adv Drug Deliv Rev. 2010;62(11):1052–63. http://dx.doi.org/10.1016/j.addr.2010.08.004
24. Lammers T, Kiessling F, Hennink WE, Storm G. Nanotheranostics and image-guided drug delivery: Current concepts and future directions. Mol Pharma. 2010;7(6):1899–912. http://dx.doi.org/10.1021/mp100228v

25. Lee DE, Koo H, Sun IC, Ryu JH, Kim K, Kwon IC. Multifunctional nanoparticles for multimodal imaging and theragnosis. Chem Soc Rev. 2012;41(7):2656–72. http://dx.doi.org/10.1039/C2CS15261D
26. Sanna V, Pala N, Sechi M. Targeted therapy using nanotechnology: Focus on cancer. Int J Nanomed. 2014;9:467–83.
27. Sahandi Zangabad P, Karimi M, Mehdizadeh F, Malekzad H, Ghasemi A, Bahrami S, et al. Nanocaged platforms: Modification, drug delivery and nanotoxicity. Opening synthetic cages to release the tiger. Nanoscale. 2017;9(4):1356–92. http://dx.doi.org/10.1039/C6NR07315H
28. Ding C, Tong L, Feng J, Fu J. Recent advances in stimuli-responsive release function drug delivery systems for tumor treatment. Molecules. 2016;21(12):pii: E1715. http://dx.doi.org/10.3390/molecules21121715
29. Chimene D, Alge DL, Gaharwar AK. Two-dimensional nanomaterials for biomedical applications: Emerging trends and future prospects. Adv Mater. 2015;27(45):7261–84. http://dx.doi.org/10.1002/adma.201502422
30. DeVita VT, Jr., Chu E. A history of cancer chemotherapy. Cancer Res. 2008;68(21):8643–53. http://dx.doi.org/10.1158/0008-5472.CAN-07-6611
31. Lacerda L, Bianco A, Prato M, Kostarelos K. Carbon nanotubes as nanomedicines: From toxicology to pharmacology. Adv Drug Del Rev. 2006;58(14):1460–70. http://dx.doi.org/10.1016/j.addr.2006.09.015
32. Zhang XY, Zhang PY. Nanotechnology for multimodality treatment of cancer. Oncol Let. 2016;12(6):4883–6.
33. Ajorlou E, Khosroushahi AY. Trends on polymer- and lipid-based nanostructures for parenteral drug delivery to tumors. Cancer Chem Pharm. 2017;79(2):251–65. http://dx.doi.org/10.1007/s00280-016-3168-6
34. Kunz-Schughart LA, Dubrovska A, Peitzsch C, Ewe A, Aigner A, Schellenburg S, et al. Nanoparticles for radiooncology: Mission, vision, challenges. Biomaterials. 2017;120:155–84. http://dx.doi.org/10.1016/j.biomaterials.2016.12.010
35. Poon W, Zhang X, Bekah D, Teodoro JG, Nadeau JL. Targeting B16 tumors in vivo with peptide-conjugated gold nanoparticles. Nanotechnology. 2015;26(28):285101. http://dx.doi.org/10.1088/0957-4484/26/28/285101
36. Moghimi SM, Farhangrazi ZS. Nanomedicine and the complement paradigm. Nanomedicine. 2013;9(4):458–60. http://dx.doi.org/10.1016/j.nano.2013.02.011
37. Weissig V, Pettinger TK, Murdock N. Nanopharmaceuticals (Part 1): Products on the market. Int J Nanomed. 2014;9:4357–73. http://dx.doi.org/10.2147/IJN.S46900
38. Bae YH, Park K. Targeted drug delivery to tumors: Myths, reality and possibility. J Control Release. 2011;153(3):198–205. http://dx.doi.org/10.1016/j.jconrel.2011.06.001
39. Balch CM, Gershenwald JE, Soong SJ, Thompson JF, Atkins MB, Byrd DR, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol. 2009;27(36):6199–206.
40. Ahlgrimm-Siess V, Laimer M, Arzberger E, Hofmann-Wellenhof R. New diagnostics for melanoma detection: From artificial intelligence to RNA microarrays. Future Oncol. 2012;8(7):819–27. http://dx.doi.org/10.2217/fon.12.84
41. Ferris LK, Harris RJ. New diagnostic aids for melanoma. Dermatol Clin. 2012;30(3):535–45. http://dx.doi.org/10.1016/j.det.2012.04.012
42. Cooper C, Sorrell J, Gerami P. Update in molecular diagnostics in melanocytic neoplasms. Adv Anat Pathol. 2012;19(6):410–16. http://dx.doi.org/10.1097/PAP.0b013e318271a5cb
43. Joyce CW, Murphy IG, Rafferty M, Ryan D, McDermott EW, Gallagher WM. Tumor profiling using protein biomarker panels in malignant melanoma: Application of tissue microarrays and beyond. Exp Rev Proteomics. 2012;9(4):415–23. http://dx.doi.org/10.1586/epr.12.5
44. Leachman SA, Cassidy PB, Chen SC, Curiel C, Geller A, Gareau D, et al. Methods of melanoma detection. Cancer Treat Res. 2016;167:51–105.
45. Kim MJ, Lee JY, Nehrbass U, Song R, Choi Y. Detection of melanoma using antibody-conjugated quantum dots in a coculture model for high-throughput screening system. Analyst. 2012;137(6):1440–5. http://dx.doi.org/10.1039/c2an16013g

46. Zheng H, Chen G, DeLouise LA, Lou Z. Detection of the cancer marker CD146 expression in melanoma cells with semiconductor quantum dot label. J Biomed Nanotechnol. 2010;6:303–11. http://dx.doi.org/10.1166/jbn.2010.1136
47. Morosini V, Bastogne T, Frochot C, Schneider R, François A, Guillemin F et al. Quantum dot–folic acid conjugates as potential photosensitizers in photodynamic therapy of cancer. Photochem Photobiol Sci. 2011;10(5):842–51. http://dx.doi.org/10.1039/c0pp00380h
48. Fernandes, B. F. et al. Immunohistochemical expression of melan-A and tyrosinase in uveal melanoma. Journal of carcinogenesis. 2007; 6(6): 100–129.
49. Kim MJ, Lee JY, Nehrbass U, Song R, Choi Y. Detection of melanoma using antibody-conjugated quantum dots in a coculture model for high-throughput screening system. Analyst. 2012;137(6):1440–5. http://dx.doi.org/10.1039/c2an16013g
50. Rizvi SB, Yildirimer L, Ghaderi S, Ramesh B, Seifalian AM, Keshtgar M. A novel POSS-coated quantum dot for biological application. Int J Nanomed. 2012;7:3915–27.
51. Lowe AC, Hunter-Ellul LA, Wilkerson MG. Nanotoxicology. Nasir A, editor. Nanotechnology in dermatology. New York: Springer; 2013. p. 231e51.
52. Bradbury MS, Phillips E, Montero PH, Cheal SM, Stambuk H, Durack JC, et al. Clinically-translated silica nanoparticles as dual-modality cancer-targeted probes for image-guided surgery and interventions. Integr Biol. 2013;5(1):74–86. http://dx.doi.org/10.1039/C2IB20174G
53. Benezra M, Penate-Medina O, Zanzonico PB, Schaer D, Ow H, Burns A, et al. Multimodal silica nanoparticles are effective cancer-targeted probes in a model of human melanoma. J Clin Invest. 2011;121(7):2768–80.
54. Friedman R. Nano dot technology enters clinical trials. J Natl Cancer Inst. 2011;103:1428–9. http://dx.doi.org/10.1093/jnci/djr400
55. Jain S, Hirst DG, O'Sullivan JM. Gold nanoparticles as novel agents for cancer therapy. Br J Radiol. 2012;85:101–13. http://dx.doi.org/10.1259/bjr/59448833
56. Astolfo A, Schultke E, Menk RH, Kirch RD, Juurlink BHJ, Hall C, et al. In vivo visualization of gold-loaded cells in mice using XRay computed tomography. Nanomed. 2013;9:284–92. http://dx.doi.org/10.1016/j.nano.2012.06.004
57. Schultke E, Menk R, Pinzer B, Astolfo A, Stampanoni M, Arfelli F, et al. Single-cell resolution in high-resolution synchrotron X-ray CT imaging with gold nanoparticles. J Synchrotron Radiat. 2014;21:242–50. http://dx.doi.org/10.1107/S1600577513029007
58. Kim C, Cho EC, Chen J, Song KH, Au L, Favazza C, et al. In vivo molecular photoacoustic tomography of melanomas targeted by bio-conjugated gold nanocages. ACS Nano. 2010;4(8):4559. http://dx.doi.org/10.1021/nn100736c
59. Meir R, Shamalov K, Betzer O, Motiei M, Horovitz-Fried M, Yehuda R, et al. Nanomedicine for cancer immunotherapy: Tracking cancer-specific T-cells in vivo with gold nanoparticles and CT imaging. ACS Nano. 2015;9(6):6363–72.
60. Anninga B, White SH, Moncrieff M, Dziewulski P, Geh JL, Klaase J, et al. Magnetic technique for sentinel lymph node biopsy in melanoma: The MELAMAG trial. Ann Surg Oncol. 2016;23(6):2070–8. http://dx.doi.org/10.1245/s10434-016-5113-7
61. Partridge SC, Kurland BF, Liu CL, Ho RJ, Ruddell A. Tumour-induced lymph node alterations detected by MRI lymphography using gadolinium nanoparticles. Sci Rep. 2015;5:15641. http://dx.doi.org/10.1038/srep15641
62. Zhou Z, Liu H, Chi X, Chen J, Wang L, Sun C, et al. A protein-corona-free T 1–T 2 dual-modal contrast agent for accurate imaging of lymphatic tumor metastasis. ACS App Mater Interf. 2015;7(51):28286–93.
63. Zhang C, Jugold M, Woenne EC, Lammers T, Morgenstern B, Mueller MM, et al. Specific targeting of tumor angiogenesis by RGD-conjugated ultrasmall superparamagnetic iron oxide particles using a clinical 1.5-T magnetic resonance scanner. Cancer Res. 2007;67(4):1555–62.
64. Melemenidis S, Jefferson A, Ruparelia N, Akhtar AM, Xie J, Allen D, et al. Molecular magnetic resonance imaging of angiogenesis in vivo using polyvalent cyclic RGD-iron oxide microparticle conjugates. Theranostics. 2015;5(5):515. http://dx.doi.org/10.7150/thno.10319

65. Huang SK, Hoon DS. Liquid biopsy utility for the surveillance of cutaneous malignant melanoma patients. Mol Oncol. 2016;10(3):450–63. http://dx.doi.org/10.1016/j.molonc.2015.12.008
66. Seenivasan R, Maddodi N, Setaluri V, Gunasekaran S. An electrochemical immunosensing method for detecting melanoma cells. Biosens Bioelectron. 2015;68:508–15. http://dx.doi.org/10.1016/j.bios.2015.01.022
67. Mi Y, Shao Z, Vang J, Kaidar-Person O, Wang AZ. Application of nanotechnology to cancer radiotherapy. Cancer Nanotechnol. 2016;7(1):11. http://dx.doi.org/10.1186/s12645-016-0024-7
68. Zhang XD, Chen J, Luo Z, Wu D, Shen X, Song SS, et al. Enhanced tumor accumulation of sub-2 nm gold nanoclusters for cancer radiation therapy. Adv Health Mater. 2014;3(1):133–41. http://dx.doi.org/10.1002/adhm.201300189
69. Maeda H. Toward a full understanding of the EPR effect in primary and metastatic tumors as well as issues related to its heterogeneity. Adv Drug Del Rev. 2015;91:3–6. http://dx.doi.org/10.1016/j.addr.2015.01.002
70. Jin W, Wang Q, Wu M, Li Y, Tang G, Ping Y, et al. Lanthanide-integrated supramolecular polymeric nanoassembly with multiple regulation characteristics for multidrug-resistant cancer therapy. Biomater. 2017;129:83–97. http://dx.doi.org/10.1016/j.biomaterials.2017.03.020
71. Gabizon AA, Patil Y, La-Beck NM. New insights and evolving role of pegylated liposomal doxorubicin in cancer therapy. Drug Resist Updat. 2016;29:90–106.
72. Wong JK, Mohseni R, Hamidieh AA, MacLaren RE, Habib N, Seifalian AM. Will nanotechnology bring new hope for gene delivery? Trends Biotechnol. 2017;35(5):434–51. http://dx.doi.org/10.1016/j.tibtech.2016.12.009
73. Bae KH, Chung HJ, Park TG. Nanomaterials for cancer therapy and imaging. Mol Cells. 2011;31(4):295–302. http://dx.doi.org/10.1007/s10059-011-0051-5
74. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. Nat Nanotechnol. 2007;2(12):751–60. http://dx.doi.org/10.1038/nnano.2007.387
75. Petros RA, DeSimone JM. Strategies in the design of nanoparticles for therapeutic applications. Nat Rev Drug Discov. 2010;9(8):615–27. http://dx.doi.org/10.1038/nrd2591
76. Dobrovolskaia MA, Aggarwal P, Hall JB, McNeil SE. Preclinical studies to understand nanoparticle interaction with the immune system and its potential effects on nanoparticle biodistribution. Mol Pharm. 2008;5(4):487–95. http://dx.doi.org/10.1021/mp800032f
77. Yang Q, Lai SK. Anti-PEG immunity: Emergence, characteristics, and unaddressed questions. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2015;7(5):655–77. http://dx.doi.org/10.1002/wnan.1339
78. Hu C-MJ, Zhang L. Nanoparticle-based combination therapy toward overcoming drug resistance in cancer. Bioch Pharmacol. 2012;83(8):1104–11. http://dx.doi.org/10.1016/j.bcp.2012.01.008
79. Allen TM, Cullis PR. Liposomal drug delivery systems: From concept to clinical applications. Adv Drug Del Rev. 2013;65(1):36–48. http://dx.doi.org/10.1016/j.addr.2012.09.037
80. Zununi Vahed S, Salehi R, Davaran S, Sharifi S. Liposome-based drug co-delivery systems in cancer cells. Mater Sci Eng C Mat Biol Appl. 2017;71:1327–41. http://dx.doi.org/10.1016/j.msec.2016.11.073
81. Guo S, Wang Y, Miao L, Xu Z, Lin C-HM, Huang L. Turning a water and oil insoluble cisplatin derivative into a nanoparticle formulation for cancer therapy. Biomaterials. 2014;35(26):7647–53. http://dx.doi.org/10.1016/j.biomaterials.2014.05.045
82. Satpathy M, Mezencev R, Wang L, McDonald JF. Targeted in vivo delivery of EGFR siRNA inhibits ovarian cancer growth and enhances drug sensitivity. Sci Rep. 2016;6:36518. http://dx.doi.org/10.1038/srep36518
83. Keles E, Song Y, Du D, Dong WJ, Lin Y. Recent progress in nanomaterials for gene delivery applications. Biomat Sci. 2016;4(9):1291–309. http://dx.doi.org/10.1039/C6BM00441E
84. Zhang XY, Zhang PY. Mitochondria targeting nano agents in cancer therapeutics. Onco Lett. 2016;12(6):4887–90. http://dx.doi.org/10.3892/ol.2016.5302
85. Hidalgo T, Gimenez-Marques M, Bellido E, Avila J, Asensio MC, Salles F, et al. Chitosan-coated mesoporous MIL-100(Fe) nanoparticles as improved bio-compatible oral nanocarriers. Sci Rep. 2017;7:43099. http://dx.doi.org/10.1038/srep43099

86. Florinas S, Liu M, Fleming R, Van Vlerken-Ysla L, Ayriss J, Gilbreth R, et al. A nanoparticle platform to evaluate bioconjugation and receptor-mediated cell uptake using cross-linked polyion complex micelles bearing antibody fragments. Biomacromolecules. 2016;17(5):1818–33.
87. Haume K, Rosa S, Grellet S, Smialek MA, Butterworth KT, Solov'yov AV, et al. Gold nanoparticles for cancer radiotherapy: A review. Can Nanotechnol. 2016;7(1):8.
88. Quyen Chau ND, Menard-Moyon C, Kostarelos K, Bianco A. Multifunctional carbon nanomaterial hybrids for magnetic manipulation and targeting. Bioch Bioph Res Commun. 2015;468(3):454–62. http://dx.doi.org/10.1016/j.bbrc.2015.06.131
89. Ahmad MW, Xu W, Kim SJ, Baeck JS, Chang Y, Bae JE, et al. Potential dual imaging nanoparticle: Gd2O3 nanoparticle. Sci Rep. 2015;5:8549. http://dx.doi.org/10.1038/srep08549
90. Zhou Z, Forbes RT, D'Emanuele A. Preparation of core-crosslinked linear-dendritic copolymer micelles with enhanced stability and their application for drug solubilisation. Int J Pharm. 2017;523(1):260–9. http://dx.doi.org/10.1016/j.ijpharm.2017.03.032
91. Orthaber K, Pristovnik M, Skok K, Perić B, Maver U. Skin cancer and its treatment: Novel treatment approaches with emphasis on nanotechnology. J Nanomat. 2017;2017:1–20.
92. Bazak R, Houri M, El Achy S, Kamel S, Refaat T. Cancer active targeting by nanoparticles: A comprehensive review of literature. J Cancer Res Clin Oncol. 2015;141(5):769–84. http://dx.doi.org/10.1007/s00432-014-1767-3
93. Sultana S, Khan MR, Kumar M, Kumar S, Ali M. Nanoparticles-mediated drug delivery approaches for cancer targeting: A review. J Drug Targ. 2013;21(2):107–25. http://dx.doi.org/10.3109/1061186X.2012.712130
94. Wang H, Lee D-K, Chen K-Y, Chen J-Y, Zhang K, Silva A, et al. Mechanism-independent optimization of combinatorial nanodiamond and unmodified drug delivery using a phenotypically driven platform technology. ACS Nano. 2015;9(3):3332–44. http://dx.doi.org/10.1021/acsnano.5b00638
95. Bertrand N, Wu J, Xu X, Kamaly N, Farokhzad OC. Cancer nanotechnology: The impact of passive and active targeting in the era of modern cancer biology. Adv Drug Del Rev. 2014;66:2–25. http://dx.doi.org/10.1016/j.addr.2013.11.009
96. Daga M, Dianzani C, Ferrara B, Nardozza V, Cavalli R, Barrera G. Latest news on nanotechnology for melanoma therapy and diagnosis. SM J Nanotechnol Nanomed. 2016;1(1):1002.
97. Li J, Wang Y, Liang R, An X, Wang K, Shen G, et al. Recent advances in targeted nanoparticles drug delivery to melanoma. Nanomedicine. 2015;11(3):769–94. http://dx.doi.org/10.1016/j.nano.2014.11.006
98. Vinogradov S, Wei X. Cancer stem cells and drug resistance: The potential of nanomedicine. Nanomedicine. 2012;7(4):597–615. http://dx.doi.org/10.2217/nnm.12.22
99. Urban C, Urban AS, Charron H, Joshi A. Externally modulated theranostic nanoparticles. Trans Cancer Res. 2013;2(4):292.
100. Chidambaram M, Manavalan R, Kathiresan K. Nanotherapeutics to overcome conventional cancer chemotherapy limitations. J Pharmacy Pharmaceut Sci. 2011;14(1):67–77. http://dx.doi.org/10.18433/J30C7D
101. Dilnawaz F, Singh A, Mohanty C, Sahoo SK. Dual drug loaded superparamagnetic iron oxide nanoparticles for targeted cancer therapy. Biomaterials. 2010;31(13):3694–706. http://dx.doi.org/10.1016/j.biomaterials.2010.01.057
102. Tran MA, Watts RJ, Robertson GP. Use of liposomes as drug delivery vehicles for treatment of melanoma. Pigment Cell Melanoma Res. 2009;22(4):388–99. http://dx.doi.org/10.1111/j.1755-148X.2009.00581.x
103. Miele, E., Spinelli, G. P., Miele, E., Tomao, F. & Tomao, S. Albumin-bound formulation of paclitaxel (Abraxane® ABI-007) in the treatment of breast cancer. International journal of nanomedicine. 2009; 4: 99–105.
104. Bei D, Meng J, Youan B-BC. Engineering nanomedicines for improved melanoma therapy: Progress and promises. Nanomed. 2010;5(9):1385–99. http://dx.doi.org/10.2217/nnm.10.117
105. Schilrreff P, Mundiña-Weilenmann C, Romero EL, Morilla MJ. Selective cytotoxicity of PAMAM G5 core–PAMAM G2. 5 shell tecto-dendrimers on melanoma cells. Int J Nanomed. 2012;7:4121–33.
106. Pegoraro C, Cecchin D, Gracia LS, Warren N, Madsen J, Armes SP, et al. Enhanced drug delivery to melanoma cells using PMPC-PDPA polymersomes. Cancer Let. 2013;334(2):328–37. http://dx.doi.org/10.1016/j.canlet.2013.02.007

107. Rahimpour Y, Hamishehkar H. Niosomes as carrier in dermal drug delivery: INTECH Open Access Publisher. Tabriz University of Medical Science, Iran; 2012.
108. Dwivedi A, Mazumder A, Du Plessis L, Du Preez JL, Haynes RK, Du Plessis J. In vitro anti-cancer effects of artemisone nano-vesicular formulations on melanoma cells. Nanomedicine. 2015;11(8):2041–50. http://dx.doi.org/10.1016/j.nano.2015.07.010
109. Chaudhuri P, Soni S, Sengupta S. Single-walled carbon nanotube-conjugated chemotherapy exhibits increased therapeutic index in melanoma. Nanotechnology. 2009;21(2):025102. http://dx.doi.org/10.1088/0957-4484/21/2/025102
110. Tomalia DA, Khanna SN. A systematic framework and nanoperiodic concept for unifying nanoscience: Hard/soft nanoelements, superatoms, meta-atoms, new emerging properties, periodic property patterns, and predictive Mendeleev-like nanoperiodic tables. Chem Rev. 2016;116(4):2705–74. http://dx.doi.org/10.1021/acs.chemrev.5b00367
111. Rej E, Gaebel T, Boele T, Waddington DE, Reilly DJ. Hyperpolarized nanodiamond with long spin-relaxation times. Nature Com. 2015;6:8459. http://dx.doi.org/10.1038/ncomms9459
112. Luk BT, Fang RH, Zhang L. Lipid-and polymer-based nanostructures for cancer theranostics. Theranostics. 2012;2(12):1117–26. http://dx.doi.org/10.7150/thno.4381
113. Wang LS, Chuang MC, Ho JA. Nanotheranostics—A review of recent publications. Int J Nanomed. 2012;7:4679–95.
114. Fraix A, Kandoth N, Manet I, Cardile V, Graziano AC, Gref R, et al. An engineered nanoplatform for bimodal anticancer phototherapy with dual-color fluorescence detection of sensitizers. Chem Commun. 2013;49(40):4459–61. http://dx.doi.org/10.1039/c3cc40714d
115. Kotb S, Detappe A, Lux F, Appaix F, Barbier EL, Tran V-L, et al. Gadolinium-based nanoparticles and radiation therapy for multiple brain melanoma metastases: Proof of concept before phase I trial. Theranostics. 2016;6(3):418. http://dx.doi.org/10.7150/thno.14018
116. Xie Z, Su Y, Kim GB, Selvi E, Ma C, Aragon-Sanabria V, et al. Immune Cell-Mediated Biodegradable Theranostic Nanoparticles for Melanoma Targeting and Drug Delivery. Small. 2017;13(10): 157–170.
117. Ciruelos E, Jackisch C. Evaluating the role of nab-paclitaxel (Abraxane) in women with aggressive metastatic breast cancer. Expert Rev Anticancer Ther. 2014;14(5):511–21. http://dx.doi.org/10.1586/14737140.2014.883922
118. Thakor AS, Gambhir SS. Nanooncology: The future of cancer diagnosis and therapy. CA Cancer J Clin. 2013;63(6):395–418. http://dx.doi.org/10.3322/caac.21199
119. Oh Y-K, Park TG. siRNA delivery systems for cancer treatment. Adv Drug Del Rev. 2009;61(10):850–62. http://dx.doi.org/10.1016/j.addr.2009.04.018
120. Rigel DS, Russak J, Friedman R. The evolution of melanoma diagnosis: 25 years beyond the ABCDs. CA Cancer J Clin. 2010;60(5):301–16. http://dx.doi.org/10.3322/caac.20074
121. Li S-Y, Liu Y, Xu C-F, Shen S, Sun R, Du X-J, et al. Restoring anti-tumor functions of T cells via nanoparticle-mediated immune checkpoint modulation. J Control Release. 2016;231:17–28. http://dx.doi.org/10.1016/j.jconrel.2016.01.044
122. Sheng W-Y, Huang L. Cancer immunotherapy and nanomedicine. Pharmaceut Res. 2011;28(2):200–14. http://dx.doi.org/10.1007/s11095-010-0258-8
123. Hong E, Usiskin IM, Bergamaschi C, Hanlon DJ, Edelson RL, Justesen S, et al. Configuration-dependent presentation of multivalent IL-15: IL-15Rα enhances the antigen-specific T cell response and anti-tumor immunity. J Biol Chem. 2016;291(17):8931–50. http://dx.doi.org/10.1074/jbc.M115.695304
124. Beloor J, Choi CS, Nam HY, Park M, Kim SH, Jackson A, et al. Arginine-engrafted biodegradable polymer for the systemic delivery of therapeutic siRNA. Biomaterials. 2012;33(5):1640–50. http://dx.doi.org/10.1016/j.biomaterials.2011.11.008
125. Ott PA, Chang J, Madden K, Kannan R, Muren C, Escano C, et al. Oblimersen in combination with temozolomide and albumin-bound paclitaxel in patients with advanced melanoma: A phase I trial. Cancer Chemother Pharmacol. 2013;71(1):183–91. http://dx.doi.org/10.1007/s00280-012-1995-7
126. Chen J, Iverson D. Estrogen in obesity-associated colon cancer: Friend or foe? Protecting postmenopausal women but promoting late-stage colon cancer. Cancer Causes Control. 2012;23(11):1767–73. http://dx.doi.org/10.1007/s10552-012-0066-z

127. Inamdar GS, Madhunapantula SV, Robertson GP. Targeting the MAPK pathway in melanoma: Why some approaches succeed and other fail. Bioch Pharmacol. 2010;80(5):624–37. http://dx.doi.org/10.1016/j.bcp.2010.04.029
128. Dhomen N, Da Rocha Dias S, Hayward R, Ogilvie L, Hedley D, Delmas V, et al. Inducible expression of V600EBraf using tyrosinase-driven Cre recombinase results in embryonic lethality. Pigment Cell Melanoma Res. 2010;23(1):112–20. http://dx.doi.org/10.1111/j.1755-148X.2009.00662.x
129. Yin D, Li Y, Lin H, Guo B, Du Y, Li X, et al. Functional graphene oxide as a plasmid-based Stat3 siRNA carrier inhibits mouse malignant melanoma growth in vivo. Nanotechnology. 2013;24(10):105102. http://dx.doi.org/10.1088/0957-4484/24/10/105102
130. Kong L-Y, Gelbard A, Wei J, Reina-Ortiz C, Wang Y, Yang EC, et al. Inhibition of p-STAT3 enhances IFN-α efficacy against metastatic melanoma in a murine model. Clin Cancer Res. 2010;16(9):2550–61. http://dx.doi.org/10.1158/1078-0432.CCR-10-0279
131. Baldea I, Filip A. Photodynamic therapy in melanoma–an update. J Physiol Pharmacol. 2012;63(2):109–18.
132. Yao H, Ng SS, Huo L-F, Chow BK, Shen Z, Yang M, et al. Effective melanoma immunotherapy with interleukin-2 delivered by a novel polymeric nanoparticle. Mol Cancer Ther. 2011;10(6):1082–92. http://dx.doi.org/10.1158/1535-7163.MCT-10-0717
133. Camerin M, Magaraggia M, Soncin M, Jori G, Moreno M, Chambrier I, et al. The in vivo efficacy of phthalocyanine–nanoparticle conjugates for the photodynamic therapy of amelanotic melanoma. Eur J Cancer. 2010;46(10):1910–18. http://dx.doi.org/10.1016/j.ejca.2010.02.037
134. Sato M, Yamashita T, Ohkura M, Osai Y, Sato A, Takada T, et al. N-propionyl-cysteaminylphenol-magnetite conjugate (NPrCAP/M) is a nanoparticle for the targeted growth suppression of melanoma cells. J Invest Dermatol. 2009;129(9):2233–41. http://dx.doi.org/10.1038/jid.2009.39
135. de Souza FF, Dos Santos MC, Dos Passos DCS, de Oliveira L, Celma E, Guillo LA. Curcumin associated magnetite nanoparticles inhibit in vitro melanoma cell growth. J Nanosci Nanotechnol. 2011;11(9):7603–10. http://dx.doi.org/10.1166/jnn.2011.5124
136. Lammers T, Kiessling F, Hennink WE, Storm G. Drug targeting to tumors: Principles, pitfalls and (pre-) clinical progress. J Control Release. 2012;161(2):175–87. http://dx.doi.org/10.1016/j.jconrel.2011.09.063
137. Neagu M, Piperigkou Z, Karamanou K, Engin AB, Docea AO, Constantin C, et al. Protein biocorona: Critical issue in immune nanotoxicology. Arch Toxicol. 2017;91(3):1031–48. http://dx.doi.org/10.1007/s00204-016-1797-5
138. Simon AK, Hollander GA, McMichael A. Evolution of the immune system in humans from infancy to old age. Proc Biol Sci. 2015;282(1821):20143085
139. Fadeel B, Fornara A, Toprak MS, Bhattacharya K. Keeping it real: The importance of material characterization in nanotoxicology. Biochem Biophys Res Commun. 2015;468(3):498–503. http://dx.doi.org/10.1016/j.bbrc.2015.06.178
140. Constantin C, Neagu M. Bio-inspired nanomaterials—A better option for nanomedicine. TTRS. 2017;1(1):3–20.
141. Nel AE, Madler L, Velegol D, Xia T, Hoek EM, Somasundaran P, et al. Understanding biophysicochemical interactions at the nano–bio interface. Nat Mater. 2009;8:543–57. http://dx.doi.org/10.1038/nmat2442
142. Karim R, Somani S, Al Robaian M, Mullin M, Amor R, McConnell G, et al. Tumor regression after intravenous administration of targeted vesicles entrapping the vitamin E α-tocotrienol. J Control Release. 2017;246:79–87. http://dx.doi.org/10.1016/j.jconrel.2016.12.014
143. Evangelopoulos M, Parodi A, Martinez JO, Yazdi IK, Cevenini A, van de Ven AL, et al. Cell source determines the immunological impact of biomimetic nanoparticles. Biomaterials. 2016;82:168–77. http://dx.doi.org/10.1016/j.biomaterials.2015.11.054
144. Dong X, Chu D, Wang Z. Leukocyte-mediated delivery of nanotherapeutics in inflammatory and tumor sites. Theranostics. 2017;7(3):751–63. http://dx.doi.org/10.7150/thno.18069
145. Engin AB, Neagu M, Golokhvast K, Tsatsakis A. Nanoparticles and endothelium: An update on the toxicological interactions. FARMACIA. 2015;63(6):792–804.

146. Li R, Ning Z, Majumdar R, Cui J, Takabe W, Jen N, et al. Ultrafine particles from diesel vehicle emissions at different driving cycles induce differential vascular pro-inflammatory responses: Implication of chemical components and NF-kappaB signaling. Part Fibre Toxicol. 2010;7:6. http://dx.doi.org/10.1186/1743-8977-7-6
147. Sarna M, Olchawa M, Zadlo A, Wnuk D, Sarna T. The nanomechanical role of melanin granules in the retinal pigment epithelium. Nanomedicine. 2016;13(3):801–7. http://dx.doi.org/10.1016/j.nano.2016.11.020
148. Koury J, Zhong L, Hao J. Targeting signaling pathways in cancer stem cells for cancer treatment. Stem Cells Int. 2017;2017:2925869.
149. Kumar D, Gorain M, Kundu G, Kundu GC. Therapeutic implications of cellular and molecular biology of cancer stem cells in Melanoma. Mol Cancer. 2017;16(1):7. http://dx.doi.org/10.1186/s12943-016-0578-3
150. Kaneti L, Bronshtein T, Malkah Dayan N, Kovregina I, Letko Khait N, Lupu-Haber Y, et al. Nanoghosts as a novel natural nonviral gene delivery platform safely targeting multiple cancers. Nano Lett. 2016;16(3):1574–82. http://dx.doi.org/10.1021/acs.nanolett.5b04237
151. Grabbe S, Haas H, Diken M, Kranz LM, Langguth P, Sahin U. Translating nanoparticulate-personalized cancer vaccines into clinical applications: Case study with RNA-lipoplexes for the treatment of melanoma. Nanomed (Lond). 2016;11(20):2723–34. http://dx.doi.org/10.2217/nnm-2016-0275
152. Kirtane AR, Sadhukha T, Kim H, Khanna V, Koniar B, Panyam J. Fibrinolytic enzyme cotherapy improves tumor perfusion and therapeutic efficacy of anticancer nanomed. Cancer Res. 2017;77(6):1465–75. http://dx.doi.org/10.1158/0008-5472.CAN-16-1646
153. Zhao J, Castranova V. Toxicology of nanomaterials used in nanomedicines. J Toxicol Environ Health B Crit Rev. 2011;14(8):593–632. http://dx.doi.org/10.1080/10937404.2011.615113
154. Satalkar P, Elger BS, Shaw DM. Stakeholder views on participant selection for first-in-human trials in cancer nanomedicine. Curr Oncol. 2016;23(6):e530–7. http://dx.doi.org/10.3747/co.23.3214

11 Short-Term and Long-Term Management of Melanoma

NEHA GOEL[1] • WILLIAM H. WARD[1] • JIAN Q. YU[2] • JEFFREY M. FARMA[1]

[1]Department of Surgical Oncology, Fox Chase Cancer Center, Philadelphia, PA, USA; [2]Department of Diagnostic Imaging, Fox Chase Cancer Center, Philadelphia, PA, USA

Author for correspondence: Neha Goel, Department of Surgical Oncology, Fox Chase Cancer Center, Philadelphia, PA, USA. E-mail: neha.goel@fccc.edu

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Abstract: Without consensus guidelines for surveillance in patients with resected melanoma, much debate exists on the appropriate short-term and long-term management of melanoma. When discussing surveillance, it is also important to keep in mind the long-term impact of ongoing surveillance in terms of improved survival, patient quality of life, cost effectiveness, and exposure to risks associated with certain surveillance methods. Most studies recommend frequent follow-up visits with dermatologic surveillance to detect potentially curable recurrence, especially resectable locoregional recurrences. Surveillance laboratory tests and chest x-rays (CXR) can have limited value while producing a relatively high false-positive rate. Lymph node ultrasonography is a valuable imaging modality in patients with equivocal lymphatic nodal basin physical examinations. In patients with early stages of melanoma, the benefit of routine surveillance imaging studies is questionable; however, close surveillance with detailed medical history and physical examination is necessary, with special attention to regional recurrences every 3–12 months, depending on the American Joint Committee on Cancer (AJCC) stage category the patient falls into and the risk of recurrence. In Stage III or greater, more frequent surveillance in the form of more frequent physical examination, laboratory tests based on symptomatology, and cross-sectional imaging

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may be indicated because of the higher risk of recurrence in this population. CT, MRI, and/or PET/CT are often a component of the overall follow-up for these high-risk patients. Additional studies are needed to better define the role of surveillance in the asymptomatic patient with resected melanoma.

Key words: Management; Melanoma; Surveillance; Survival

Introduction

In the absence of consensus guidelines for surveillance in patients with resected melanoma, much debate exists on the appropriate short-term and long-term management of melanoma (1). When discussing surveillance, it is also important to keep in mind the long-term impact of ongoing surveillance in terms of improved survival, patient quality of life, cost effectiveness, and exposure to risks associated with certain surveillance methods (2). Some studies recommend frequent follow-up visits with abundant use of radiographic imaging and laboratory review, while others question the value of these strategies altogether (3, 4)

According to the National Comprehensive Cancer Network (NCCN), the lifetime risk of developing a second primary melanoma approaches 4–8%; therefore, lifetime dermatological surveillance is recommended (1). However, follow-up recommendations vary worldwide and guidelines are disparate. Lifelong surveillance is important because of the risk of (i) second primary melanomas, (ii) locoregional recurrence, (iii) late recurrence, and (iv) other cutaneous malignancies. The risk of local recurrence is greatest in the first 5 years after diagnosis, especially in thick and ulcerated tumors (5). Locoregional recurrence of melanoma is defined as recurrence at the site of the primary lesion, regionally in the draining lymph node basin, or in between. Satellite and in-transit metastases are differentiated by their distance from the primary site, with satellite lesions occurring within 2 cm and in-transit metastases occurring more than 2 cm from the primary lesion. Both satellite and in-transit metastases are considered Stage IIIB (without regional nodal metastases) or Stage IIIC (with regional nodal metastases) disease. (American Joint Committee on Cancer 7th edition) (6).

Dermatological Surveillance

TOTAL CUTANEOUS EXAMINATION

Dermatologic surveillance includes a total-body skin examination, palpation of the primary site and surrounding area for local recurrences, satellitosis, in-transit metastases, and a thorough regional lymph node basin examination. In addition, a review of systems should include questions about new or changing lesions, weight loss, fatigue, headache, new back pain, and any new symptoms that have persisted. Patients should be counseled to adhere to sun-protective measures and perform skin self-examinations.

Regular skin surveillance with monthly self-examination and total cutaneous examinations (TCEs) by a dermatologist increases the chances of detecting

melanomas when they are thinner, thereby reducing morbidity and mortality. However, there are no controlled trials evaluating TCE on melanoma mortality. Berwick et al. describes an association between regular skin self-examination and reduction in the risk of developing advanced melanoma, reducing melanoma mortality by 63% (7). De Giorgi et al. studied 802 patients retrospectively and found that 36% of melanomas were discovered during annual TCEs by dermatologist and 33% were discovered by patients. Additional analysis showed that self-detection was linked with a greater probability of having a thicker melanoma (8).

In 1987, a follow-up protocol at the Yale Melanoma Unit was devised to improve the detection of recurrence in patients with Stage I–Stage III melanoma. The protocol included a patient education program and a standardized follow-up schedule. A retrospective evaluation of 419 patients treated from January 1988 to December 1994 revealed that of the 78 patients with disease recurrence, 44% had clinical symptoms initially detected by the patients and 56% of recurrences were detected by physician-directed surveillance examinations (9). Most recurrences were found within the first (47%) or second (32%) year of follow-up. The study results recommended the following surveillance guidelines: (i) Stage I, annually; (ii) Stage II, every 6 months for years 1–2 and annually thereafter; (iii) Stage III, every 3 months for year 1, every 4 months for year 2, and every 6 months for years 3–5; (iv) at year 6 and beyond, all patients should have surveillance annually, due to the risk of late recurrence and/or multiple primaries (9).

Garbe et al. prospectively analyzed 2008 patients within a single institution in Germany. A total of 233 metastatic recurrences and 62 second primary melanomas were discovered during the 25-month study period. Over 70% of recurrences were found on scheduled follow-up examinations and 17% of all recurrences were first discovered by the patients. Physical examination diagnosed 50% of recurrences and the remaining 50% were identified radiographically (10). Garbe et al. also classified recurrences as early or late in terms of development. Patients diagnosed in the early phase had significantly more favorable odds of recurrence-free and overall survival than those in a late phase.

The Scottish Melanoma Group found that almost half (47%) of recurrences were first observed by the patient, with only 26% initially detected on follow-up (11). A recent German nationwide study prospectively analyzed 668 patients from 67 centers, of whom 96% were in regular melanoma surveillance. Of the patients, 118 (11.1%) had tumor progression and the rate of progression increased with stage. However, it was higher in Stage IIC than Stage IIIA and Stage IIIB (54.2% vs. 42.9% and 43.6%, respectively). Median progression-free survival (PFS) of Stage IIC patients was 34.5 months. The rate of progression was highest in Stage IV disease (63.6%, median PFS 5.3 months). In years 3 and 4 of surveillance, 55.6% of locoregional and 60% of distant metastases were detected on regular follow-up. Only 33.3% of locoregional metastases were patient detected, although 47.2% were described as being clinically visible and 22.2% palpable. Overall, the authors questioned the benefit of frequent follow-up visits in the low-risk patient group, especially since most recurrences were locoregional and amenable to visual or palpation by the patient. Consequently, the authors recommend reducing melanoma follow-up in low-risk melanoma patients and increasing patient education in terms of how to perform self-examinations (12).

The German Cancer Society and German Dermatologic Society guidelines are stage and Breslow specific and include examination by TCE every 6 months for 5 years in Stage I with ≤1 mm thickness, every 3 months for 5 years in Stage I and Stage II with >1 mm thickness, and every 3 months for the first 3 years for Stage III. For years 6–10, the TCE is every 12 months in Stage I with ≤1 mm thickness, every 6 months in Stage I and Stage II with >1 mm thickness, and every 6 months for Stage III (10).

The Swiss guidelines are stage specific and consist of a TCE every 3 months for years 1–3, every 6 months for years 4–5, and then every 6–12 months for years 6–10 in Stage I (T2N0)–IIB patients. In Stage IIC–Stage III, TCE should be performed every 3 months for years 1–5, then every 6 months for years 6–10. They recommend individualized follow-up in patients with Stage IV disease (13).

The European Society for Medical Oncology (ESMO) guidelines do not follow a staging system but provide general recommendations for monitoring patients at risk for recurrent and new disease. The guidelines recommend that for low-risk thin melanomas imaging is not recommended and for high-risk patients (i.e., those with thick primary tumors or recent tumor resection), computed tomography (CT) and/or PET scans are suggested for earlier detection of relapse. The ESMO also recommends patient education regarding sun avoidance and lifelong regular self-examinations of the skin and peripheral lymph nodes (14).

The American Academy of Dermatology (AAD) recommends TCE at least annually and possibly every 3–12 months based on tumor stage, history of multiple melanomas, presence of atypical nevi, family history of melanoma, patient anxiety, and the patient's ability to recognize signs and symptoms of a disease. The AAD also recommends patient education on monthly self-skin and self-lymph node examinations (15).

The British Association of Dermatologists (BAD) follow-up recommendations for *in situ* melanomas are self-examination with no additional follow-up required. For Stage IA melanomas, they recommend TCE 2–4 times a year for the first year. For Stage IB to Stage IIIA melanomas, the BAD guidelines recommend a TCE every 3 months for 3 years, and then every 6 months for 2 years. For Stage IIIB and Stage IIIC and resected Stage IV melanomas, the BAD guidelines recommend evaluation every 3 months for 3 years, then every 6 months for the next 2 years, and then annually for the next 5 years. For unresected Stage IV melanomas, follow-up should be done on an individualized basis. In addition, they do not have specific guidelines for lab work or imaging (16).

Guidelines for the Management of Melanoma in Australia and New Zealand (GMMANZ) emphasize the importance of self-examinations in patients properly trained to detect recurrent disease. Along with this cost-effective measure, patients with Stage I melanoma should undergo TCE every 6 months for 5 years from a health care professional of their choice. Patients with Stage II and Stage III disease should have a TCE every 3–4 months for 5 years and annually thereafter. The guidelines recommend ultrasound (US) by an experienced US technician as the only imaging modality in patients with advanced disease. They do not have any specific recommendations for Stage IV disease. In addition, more frequent visits are recommended in patients with extensive disease, many atypical nevi, a family history of melanoma, and those with difficulty performing a self-evaluation.

GMMANZ also emphasizes the importance of evaluating individual patient needs in developing a follow-up schedule (17).

According to NCCN guidelines, the recommended follow-up is annually for Stage 0 and every 6–12 months for the first 5 years for Stage IA–Stage IIA. For Stage IIB– Stage IV, follow-up is recommended every 3–6 months for the first 2 years, then every 3–12 months for the next 3 years, and then annually starting after 6 years (1). The AAD guidelines for follow-up of resected melanoma states that no clear data regarding follow-up interval exist and that annual examinations with self-examination at regular intervals are necessary (15). As it can be seen, there is no international consensus on surveillance guidelines. Table 1 summarizes the major recommendations for follow-up examinations currently published.

TABLE 1 Guidelines for Follow-Up

Organization	Stage/Breslow thickness	History and physical	Imaging	Lab values
NCCN (National Comprehensive Cancer Network)	Stage 0	Annual for life	None	
	Stage IA–Stage IIA	Every 3–12 months for 5 years, then annually unless clinically indicated	None	
	Stage IIB–Stage IV	Every 3–6 months for 2 years, then Every 3–12 months for 3 years, then annually unless clinically indicated	CXR, CTC/A/P ± PET/CT Every 3–12 months and annual brain MRI, or as clinically indicated	
ESMO (European Society for Medical Oncology)	Thin/low risk	No specific recommendations	None	
	Thick/high risk	No specific recommendations	CTC/A/P ± PET/CT	
AAD (American Academy of Dermatology)	N/A	H and P at least annually, possibly Every 3–12 months	Not recommended in asymptomatic patients Imaging not recommended after 5 years in high-risk patients	None

Table continued on following page

TABLE 1 Guidelines for Follow-Up (Continued)

Organization	Stage/Breslow thickness	History and physical	Imaging	Lab values
BAD (British Association of Dermatologists)	*In situ* Stage IA	Self-exam, H and P Every 3–6 months for 1 year	None	
	Stage IA–Stage IIIA	H and P Every 3 months for 3 years, then Every 6 months for 2 years	None	
	Stage IIIB–Stage IV (resected)	H and P Every 3 months for 3 years, then Every 6 months for 2 years, then annually for the next 5 years	Consider CT	
	Stave IV (unresected)	As needed	No specific guidelines	
German Cancer Society and German Dermatologic Society	Stage I < 1 mm	H and P Every 6 months for the first 5 years, then Every 6–12 months for the next 5 years until year 10	None	None
	Stage I and Stage II >1 mm	H and P Every 3 months for the first 5 years, then Every 6–12 months for the next 5 years until year 10	Lymph Node US Every 6 months for years 1–5 Abdominal US and CXR on individual basis	
	Stage III	H and P Every 3 months for 5 years, and then Every 6 months for the next 5 years until year 10	Lymph Node US Every 3–6 months for years 1–5 Abdominal US and CXR on individual basis	S100β level every 3–6 months for years 1–5
	Stage IV		Abdominal US and CXR or CT, MRI, or PET Every 6 months for years 1–5	S100β level every 3–6 months for years 1–5

Table continued on following page

TABLE 1 Guidelines for Follow-Up (Continued)

Organization	Stage/Breslow thickness	History and physical	Imaging	Lab values
Swiss guidelines				
	Stage I (T2N0)–IIB	H and P Every 3 months for years 1–3, Every 6 months for years 4–5, and then Every 6–12 months for years 6–10	Lymph Node US Every 6–12 months for years 1–5	S100β Every 6–12 months for years 1–5
	Stage IIC–Stage III	H and P Every 3 months for years 1–5, then Every 6 months for years 6–10	Lymph Node US Every 6 months for years 1–5	S100β Every 6 months for years 1–5
	Stage IV	Individualized follow-up		
GMMANZ (Guidelines for the Management of Melanoma in Australia and New Zealand)	Stage I	H and P Every 6 months for 5 years		
	Stage II and Stage III	H and P Every 3–4 months for 5 years, and annually thereafter	Lymph Node US in advanced disease	
	Stage IV	No guidelines		

TOTAL CUTANEOUS PHOTOGRAPHY

Total cutaneous photography (TCP) was initially described in 1988 by William Slue as a method of taking total-body photographs to document dysplastic nevi. These photos are then reviewed and compared at subsequent follow-up examinations. Detection of thin malignant melanomas in a curable stage is enhanced by utilizing these baseline photographs (18). Currently, TCP has evolved into a system involving digital photography-based mole mapping. Patients at high risk with multiple nevi can use the photographs to assist in self-examinations. Feit et al. reported an increase in the melanoma diagnosis rate with the use of this technique. Moreover, they reported that melanomas identified with the assistance of TCP are

generally thin melanomas (19). Barriers to the increased use of TCP include the cost, which tends not to be covered by insurances, having the photos available during physical examinations, and a medical-legal concern for the potential of these photographs to be used in malpractice suits (20).

Laboratory Tests

The two potential tumor markers that exist for melanoma include lactate dehydrogenase (LDH) and S100β. LDH is found throughout the body and is expressed by a multitude of cancers and nonmalignant etiologies; however, it is unsuitable for use in screening for or diagnosis of melanoma. Persistent or recurrent elevations of LDH after treatment of melanoma may indicate residual or recurrent disease. Another marker is serum protein S100β which was first described in 1980 in cultured melanoma cells and is an immunohistochemical marker of pigmented skin lesions.

Finck et al. reported 121 Stage II and 58 Stage III patients where high levels of LDH indicated recurrence with a sensitivity and specificity of 72 and 97%, respectively. As an indicator of liver metastasis, LDH had a sensitivity and specificity of 95 and 82%, respectively, in Stage II melanoma, and 86 and 57%, respectively, in Stage III melanoma. An elevated LDH was the first indication of recurrent disease in 11/88 (12.5%) Stage II patients. The mean survival following LDH elevation was 5.9 months. It was concluded that monitoring LDH can provide useful information in the postoperative follow-up of patients with melanoma (20). Other reports have documented an association between serum levels of LDH and prognosis in patients with Stage IV melanoma; however, the prognostic value of LDH in patients with Stage I–Stage III melanoma is very limited as it is rarely elevated (21).

In a retrospective analysis of 261 patients with a regimented follow-up schedule, 145 evaluable patients developed recurrent melanomas. A total of 99 patients (68%) developed clinical symptoms that initiated a workup for recurrence. Physical examination of asymptomatic patients led to the diagnosis of recurrent disease in 37 patients (26%). The other nine patients (6%) with recurrent disease had abnormal CXR. Laboratory results were never a sole indicator of recurrent disease. They concluded blood analyses and CXR have limited value in the follow-up of patients with resected intermediate-risk and high-risk melanomas (22).

Garbe et al. evaluated 1492 patients of which 2719 blood tests (including blood count, erythrocyte sedimentation rate, renal function, liver enzymes, LDH, and S100β) were performed annually in the earlier stages and twice yearly in patients with more advanced stage melanoma. Blood tests were rarely the first sign of metastasis, and a diagnosis was made in only three patients after the detection of an elevated LDH. In patients developing metastasis, LDH and alkaline phosphatase (AP) were found to be elevated in 16.4 and 12.5%, respectively. Both percentages were significantly higher than in patients without metastasis (4.2% for LDH and 3.5% for AP, $P < 0.0001$). Half of these patients with Stage II and Stage III disease expressed serum protein S100β and it was elevated in approximately 50% of patients with distant metastasis. In patients with locoregional recurrence, only a few were found to have an elevated protein S100β (10).

Routine blood tests contribute to the detection of metastasis in a very small subset of patients. Nevertheless, increasing values of both markers, LDH and serum protein S100β, may be the first sign of recurrence. Future investigations are needed to clarify whether protein S100β is a suitable substitute for the other blood values or whether it should be used as a supplementary examination method. Currently, use of laboratory tests in the surveillance of earlier stage melanoma is not recommended.

Imaging

Currently, there are no formal imaging guidelines for surveillance in patients with resected melanomas. According to the NCCN, additional radiological imaging is only recommended based on symptoms (1). CXR, CT, and/or positron emission tomography/CT (PET/CT) are considered optional and should be tailored to the stage and discretion of the physician (1). Guidelines recommend "considering" radiological studies every 4–12 months in Stage IIB or greater (1). Published guidelines for the management of cutaneous melanoma in the United Kingdom, the Netherlands, and Australia do not recommend routine radiological investigations; however, German guidelines recommend cross-sectional imaging every 6 months for Stage IIC or greater for the first 3 years after resection. Swiss guidelines recommend annual CXRs for the first 5 years in patients with Stage I/Stage II disease, and PET/CT or CT in the follow-up of Stage III patients (22, 23).

CHEST X-RAY

A common site of distant spread for melanoma is to the lungs. Surveillance CXRs have a high number of false-positive and false-negative findings. Morton et al. studied the accuracy of surveillance CXRs and the impact on survival by evaluating the extent of distant disease, time to detection, and treatment in those with CXR-detected compared with symptomatic pulmonary metastases. A total of 108 high-risk patients were followed with CXR every 6 months for 8 years and then annually until 10 years. A total of 23 out of 108 (21%) high-risk patients developed pulmonary metastases but only 10% were detected by CXR. Sensitivity and specificity of surveillance CXRs were 48 and 78%, respectively, with a high false-positive rate. Only 3 of the 23 (13%) cases of identified pulmonary metastases were amenable to surgical intervention (22). Leiter et al. showed a benefit in the use of CXR only in Stage III disease. This study prospectively followed 1969 patients and only 10 of the 204 relapses were discovered by CXR. The majority (7/10) of recurrences were in patients with Stage III disease (24). Brown et al. reported a low sensitivity of 7.7% and a specificity of 96.5%. In a trial of 1235 patients, 210 relapses occurred, 38 of which were detected by CXR. In order to detect these 38 recurrences, a total of 4218 (38/4218, 0.9%) x-rays were performed with a 129 (3.1%) false-positive rate. Isolated pulmonary metastases amenable to resection were found in only 3 of the 38 patients (25).

In conclusion, CXR does not dependably identify pulmonary metastases, nor has it lead to earlier detection of pulmonary metastases. In most series, when pulmonary metastases are detected, they are generally unresectable. Frequent CXR

surveillance can cause unnecessary patient anxiety, given high false-positive rates as well as the significant medical costs involved.

LYMPH NODE ULTRASONOGRAPHY

Ultrasonography examines the surgical scar of the primary tumor, the in-transit area, the locoregional lymph nodes, and potentially further lymph node basins. However, its utility is user dependent. Lymph node US has been debated in terms of its efficacy in early detection of locoregional lymph node metastases (25, 26). According to a meta-analysis by Bafounta et al. of 6642 patients and 18,610 paired palpation and US examinations, US had a higher discriminatory power (odds ratio 1755; 95% CI 726–4238) than did palpation (21 [4–111]; $P = 0.0001$). Furthermore, positive-likelihood ratios were 41.9 for ultrasonography and 4.55 for palpation; negative-likelihood ratios were 0.024 and 0.22, respectively. This group concluded that US detects lymph node invasion more accurately than palpation and should therefore probably be used routinely in patients with melanoma (27). In addition, Garbe et al. reported 71% early detection compared to 48% early detection for all examination methods (10).

On the other hand, Chai et al. reviewed 325 patients with melanoma who underwent US before sentinel lymph node biopsy (SLNB) from 2005 to 2009. A total of 471 basins were examined with US. Only six patients (1.8%) avoided SLNB by undergoing US-guided fine-needle aspiration of involved nodes, followed by therapeutic lymphadenectomy. Overall, sensitivity of US was 33.8%, specificity 85.7%, positive predictive value 36.5%, and negative predictive value 84.2%. Sensitivity and specificity improved somewhat with increasing Breslow depth. Sensitivity was highest for the neck, but specificity was highest for the inguinal lymph nodes. The authors concluded that routine preoperative US in clinically node-negative melanoma is impractical because of its low sensitivity, but selected patients with thick or ulcerated lesions may benefit. However, because of variable lymphatic drainage patterns, preoperative US without lymphoscintigraphic localization will provide incomplete evaluation in many cases (28). These data can be extrapolated for patients in the follow-up setting given the low sensitivity of US in clinically node-negative patients.

Machet et al. from France performed US follow-up for 373 patients for melanomas with thick melanomas, greater than 1.5 mm, every 6 months and every year for thin melanomas, less than 1.5 mm. In total, 1909 US examinations combined with clinical examination were analyzed. Node biopsy was performed in 65 patients and demonstrated melanoma metastases in 54. Sensitivity of clinical examination and US examination was 71.4 and 92.9%, respectively. Specificity of clinical examination and US examination was 99.6 and 97.8%, respectively. Despite this apparent superiority of US examination over palpation, only 7.2% of the patients really benefited from US examination (earlier lymph node metastasis detection or avoidance of unnecessary surgery), while 5.9% had some deleterious effect from US examination such as unnecessary stress caused by repetitive US and excision of benign lymph nodes. This French group confirmed the greater sensitivity of US examination to clinical examination in the diagnosis of nodal metastases from cutaneous melanoma. However, they concluded that the role of US in routine follow-up is still questionable since only a very small

proportion of patients (1.3%) benefited from adding US to clinical examination. A large prospective randomized clinical trial would be needed to study the efficacy of US (29).

While lymph node ultrasonography has been studied, neither the NCCN nor the AAD include this technique in their recommendations. The NCCN states lymph node US may be considered in patients with an equivocal physical examination, in patients who were offered SLNB but refused, or patients with positive sentinel lymph nodes who did not receive complete lymph node dissections (1). German melanoma guidelines however do recommend lymph node ultrasonography every 6 months in Stage IB to Stage IIB and every 3 months for Stage IIC or greater (23).

COMPUTED TOMOGRAPHY/MAGNETIC RESONANCE IMAGING

Magnetic resonance imaging (MRI) can more readily detect cerebral metastases over CT and PET/CT (30). MRI has proven to be more sensitive and specific in the detection of soft tissue and osseous metastases as well (31), but there is no strong data directly comparing MRI to CT in osseous metastasis (32). Whole-body CT is a sensitive procedure, which allows for the detection of metastases as small as 2–4 mm (31). In a study by Romano et al., 72% of asymptomatic distant metastases were discovered by CT scans (3), while other trials yielded detection rates of 15–28% (10). During follow-up of patients with Stage IV disease and in cases of suspected metastasis, CT plays a pivotal role. More than 50% of recurrences in asymptomatic Stage III patients are detected by the patient or by examinations; therefore, cross-sectional imaging screening should only be performed in high-risk patients (3, 10, 33). CT has a higher sensitivity compared to MRI in the diagnosis of small pulmonary metastases (66.9 vs. 2.9%, $P < 0.0001$) and should be considered (31). Drawbacks to CT are its limited soft tissue contrast, cost, and radiation exposure.

POSITRON EMISSION TOMOGRAPHY/COMPUTED TOMOGRAPHY

PET/CT displays the uptake of radioactively labeled glucose in metabolically active areas. In a meta-analysis evaluating imaging modalities in surveillance of melanoma patients, PET/CT revealed a high sensitivity (80%) and specificity (87%) in the detection of distant metastases, higher than conventional CT (51 and 69%, respectively) (26). Rinne et al. studied 100 patients prospectively and found an increase in sensitivity from 20 to 71.4% when comparing conventional diagnostic techniques to PET/CT (30). The NCCN recommends considering PET/CT every 4–12 months in Stage IIB or higher melanoma patients (1). According to the AAD, surveillance imaging studies in asymptomatic patients have low yield for detection of metastases and are associated with high false-positive rates (15). Overall, a general recommendation on imaging procedures cannot be made based on current data as the studies included inhomogeneous patients groups and are characterized by low evidence levels. In addition, the safety of CT and PET/CT is of significant concern since large-population-based studies have shown an increased risk of cancer with cumulative radiation exposure from repeat CT and PET/CTs (34, 35).

Conclusion

The major benefit of dermatological surveillance is the detection of potentially curable recurrence, especially resectable locoregional recurrences. Surveillance laboratory tests and CXRs can have limited value while producing a relatively high false-positive rate. Lymph node ultrasonography is a valuable imaging modality in patients with equivocal lymphatic nodal basin physical examinations. In patients with early stages of melanoma, the benefit of routine surveillance imaging studies is questionable, and we do not generally perform this at our institution; however, close surveillance with detailed medical history and physical examination is necessary, with special attention to regional recurrences every 3–12 months, depending on the AJCC stage category the patient falls into and the risk of recurrence. In Stage III or greater, more frequent surveillance in the form of more frequent physical examination, laboratory tests based on symptomatology, and cross-sectional imaging may be indicated because of the higher risk of recurrence in this population. CT, MRI, and/or PET/CT are often a component of the overall follow-up for these high-risk patients. Additional studies are needed to better define the role of surveillance in the asymptomatic patient with resected melanoma.

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References

1. National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology – v.1.2017: Melanoma. Available from: http://www.nccn.org/professionals/physician_gls/pdf/melanoma.pdf
2. Brenner DJ, Hall EJ. Computed tomography—An increasing source of radiation exposure. N Engl J Med. 2007;357(22):2277–84. http://dx.doi.org/10.1056/NEJMra072149
3. Romano E, Scordo M, Dusza SW, Coit DG, Chapman PB. Site and timing of first relapse in stage III melanoma patients: Implications for follow-up guidelines. J Clin Oncol. 2010;28(18):3042–7. http://dx.doi.org/10.1200/JCO.2009.26.2063
4. Crowley NJ, Seigler HF. Late recurrence of malignant melanoma. Ann Surg 1990; 212(2): 173–177.
5. Bolognia JL, Jorizzo J, Schaffer JV. Dermatology. 3rd ed. Elsevier Limited: Saunders; 2012. p. 1905–1910.
6. Edge SB, Compton CC, Byrd DR, eds. AJCC cancer staging manual. 7th ed. New York: Springer-Verlag.
7. Berwick M, Begg CB, Fine JA, Roush GC, Barnhill RL. Screening for cutaneous melanoma by skin self-examination. J Natl Cancer Inst. 1996;88(1):17–23. http://dx.doi.org/10.1093/jnci/88.1.17
8. De Giorgi V, Grazzini M, Rossari S, Gori A, Papi F, Scarfi F, et al. Is skin self-examination for cutaneous melanoma detection still adequate? A retrospective study. Dermatology. 2012;225(1):31–6. http://dx.doi.org/10.1159/000339774
9. Poo-Hwu WJ, Ariyan S, Lamb L, Papac R, Zelterman D, Hu GL, et al. Follow-up recommendations for patients with American Joint Committee on Cancer stages I–III malignant melanoma. Cancer. 1999;86(11):2252. http://dx.doi.org/10.1002/(SICI)1097-0142(19991201)86:11%3C2252::AID-CNCR12%3E3.0.CO;2-Q

10. Garbe C, Paul A, Kohler-Spath H, Ellwanger U, Stroebel W, Schwarz M, et al. Prospective evaluation of a follow-up schedule in cutaneous melanoma patients: Recommendations for an effective follow-up strategy. J Clin Oncol. 2003;21(3):520–9. http://dx.doi.org/10.1200/JCO.2003.01.091
11. Dicker TJ, Kavanagh GM, Herd RM, Ahmad T, McLaren KM, Chetty U, et al. A rational approach to melanoma follow-up in patients with primary cutaneous melanoma. Br J Dermatol. 1999;140(2):249–54. http://dx.doi.org/10.1046/j.1365-2133.1999.02657.x
12. Livingstone E, Krajewski C, Eigentler TK, Windemuth-Kieselbach C, Benson S, Elsenbruch S, et al., Prospective evaluation of follow-up in melanoma patients in Germany—Results of a multicentre and longitudinal study. Eur J Cancer. 2015;51(5):653–67. http://dx.doi.org/10.1016/j.ejca.2015.01.007
13. Dummer R, Guggenheim M, Arnold AW, Braun R, von Moos R, Project Group Melanoma of the Swiss Group for Clinical Cancer Research. Updated Swiss guidelines for the treatment and follow-up of cutaneous melanoma. Swiss Med Wkly. 2011;141:w13320. http://dx.doi.org/10.4414/smw.2011.13320
14. Dummer R, Hauschild A, Guggenheim M, Jost L, Pentheroudakis G, ESMO Guidelines Working Group. Melanoma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2010;21(Suppl 5):v194–7. http://dx.doi.org/10.1093/annonc/mdq188
15. Bichakjian CK, Halpern AC, Johnson TM, Foote Hood A, Grichnik JM, Swetter SM, et al. Guidelines of care for the management of primary cutaneous melanoma. American Academy of Dermatology. J Am Acad Dermatol. 2011;65(5):1032–47. http://dx.doi.org/10.1016/j.jaad.2011.04.031
16. Marsden JR, Newton-Bishop JA, Burrows L, Cook M, Corrie PG, Cox NH, et al. Revised U.K. guidelines for the management of cutaneous melanoma 2010. Br J Dermatol. 2010;163(2):238–56. http://dx.doi.org/10.1111/j.1365-2133.2010.09883.x
17. Clinical practice guidelines for the management of melanoma in Australia and New Zealand. 2008. Available from: https://www.cancer.org.au/content/pdf/HealthProfessionals/ClinicalGuidelines/ClinicalPracticeGuidelines-ManagementofMelanoma.pdf
18. Slue W, Kopf AW, Rivers JK. Total-body photographs of dysplastic nevi. Arch Dermatol 1988;124(8):1239–43. http://dx.doi.org/10.1001/archderm.1988.01670080051017
19. Feit NE, Dusza S, Marghoob AA. Melanomas detected with the aid of total cutaneous photograph. Br J Dermatol. 2004;150(4):706–14. http://dx.doi.org/10.1111/j.0007-0963.2004.05892.x
20. Halpern AC. The use of whole body photography in a pigmented lesion clinic. Dematol Surg. 2000;26(12):1175–1180.
21. Finck SJ, Giuliano A, Morton DL. LDH and melanoma. Cancer. 1983;51(5):840–3. http://dx.doi.org/10.1002/1097-0142(19830301)51:5%3C840::AID-CNCR2820510516%3E3.0.CO;2-7
22. Morton RL, Craig J, Thompson JF. The role of surveillance chest x-rays in the follow-up of high-risk melanoma patients. Ann Surg Oncol. 2009;16(3):571–7. http://dx.doi.org/10.1245/s10434-008-0207-5
23. Pflugfelder A, Kochs C, Blum A, Capellaro M, Czeschik C, Dettenborn T, et al. Malignant melanoma S3-guideline "Diagnosis, therapy and follow-up of melanoma." J Dtsch Dermatol Ges. 2013;11(6):1–116. http://dx.doi.org/10.1111/ddg.12113_suppl
24. Leiter U, Marghoob A, Lasithiotakis K. Costs of the detection of metastases and follow-up examinations in cutaneous melanoma. Melanoma Res. 2009;19(1):50–7. http://dx.doi.org/10.1097/CMR.0b013e32831bc41c
25. Brown RE, Stromberg A, Hagendoorn LJ, Hulsewede DY, Ross MI, Noyes RD, et al. Surveillance after surgical treatment of melanoma: Futility of routine chest radiography. Surgery. 2010;148(4):711–16. http://dx.doi.org/10.1016/j.surg.2010.07.042
26. Xing Y, Bronstein Y, Ross MI, Askew RL, Lee JE, Gershenwald JE, et al. Contemporary diagnostic imaging modalities for the staging and surveillance of melanoma patients: A meta-analysis. J Natl Cancer Inst. 2011;103(2):129–42. http://dx.doi.org/10.1093/jnci/djq455
27. Bafounta ML, Beauchet A, Chagnon S, Saiag P. Ultrasonography or palpation for detection of melanoma nodal invasion: A meta-analysis. Lancet Oncol. 2004;5(11):673–80. http://dx.doi.org/10.1016/S1470-2045(04)01609-2
28. Chai CY, Zager JS, Szabunio MM, Marzban SS, Chau A, Rossi RM, et al. Preoperative ultrasound is not useful for identifying nodal metastasis in melanoma patients undergoing sentinel node biopsy: Preoperative ultrasound in clinically node-negative melanoma. Ann Surg Oncol. 2012;19(4):1100–6. http://dx.doi.org/10.1245/s10434-011-2172-7

29. Machet L, Nemeth-Normand F, Giraudeau B, Perrinaud A, Tiguemounine J, Ayoub J, et al. Is ultrasound lymph node examination superior to clinical examination in melanoma follow-up? A monocentre cohort study of 373 patients. Br J Dermatol. 2005;152(1):66–70. http://dx.doi.org/10.1111/j.1365-2133.2004.06262.x
30. Rinne D, Baum RP, Hor G, Kaufmann R. Primary staging and follow-up of high risk melanoma patients with whole-body 18F-fluorodeoxyglucaose positron emission tomography. Cancer. 1998;82(9):1664–71. http://dx.doi.org/10.1002/(SICI)1097-0142(19980501)82:9%3C1664::AID-CNCR11%3E3.0.CO;2-2
31. Hausmann D, Jochum S, Utikal J, Hoffmann RC, Zechmann C, Neff KW, et al. Comparison of the diagnostic accuracy of whole-body MRI and whole-body CT in stage III/IV malignant melanoma. J Dtsch Dermatol Ges. 2011;9(3):212–22. http://dx.doi.org/10.1111/j.1610-0387.2011.07614.x
32. Pfannenberg C, Aschoff P, Schanz S, Eschmann SM, Plathow C, Eigentler TK, et al. Prospective comparison of 18F-fluorodeoxyglucose positron emission tomography/computed tomography and whole-body magnetic resonance imaging in staging of advanced malignant melanoma. Eur J Cancer. 2007;43(3):557–64. http://dx.doi.org/10.1016/j.ejca.2006.11.014
33. Francken AB, Bastinaannet E, Hoekstra HJ. Follow-up in patients with localised primary cutaneous melanoma. Lancet Oncol. 2005;6(8):608–21. http://dx.doi.org/10.1016/S1470-2045(05)70283-7
34. Pearce MS, Salotti JA, Little MP, McHugh K, Lee C, Kim KP, et al., Radiation exposure from CT scans in childhood and subsequent risk of leukaemia and brain tumours: A retrospective cohort study. Lancet. 2012;380(9840):499–505. http://dx.doi.org/10.1016/S0140-6736(12)60815-0
35. Fazel R, Krumholz HM, Wang Y, Ross JS, Chen J, Ting HH, et al., Exposure to low-dose ionizing radiation from medical imaging procedures. N Engl J Med. 2009;361(9):849–57. http://dx.doi.org/10.1056/NEJMoa0901249

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