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# Roadmap for New Opportunities in Melanoma Research

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Investigators representing all major melanoma research areas present an overview of the most important challenges for the field. Four major research areas are covered plus the training of new investigators. For each area we first describe the present status, its strengths and weaknesses, and then outline specific recommendations. In basic research of melanoma, we outline the pertinent issues for melanoma classification, understanding melanocyte development and transformation, melanoma resistance, tumor microenvironment, metastasis, animal models, immune response, and blood and tissue diagnostics. In clinical research we provide an overview of the current challenges and the strategies for characterization, monitoring, and therapy. It will be important to develop strong research and clinical infrastructures by establishing tumor banks, identifying and validating biomarkers, developing new imaging techniques, and increasing multidisciplinary collaboration and communication. To strengthen the field we need to recruit both young and established investigators and foster career development plans that cover all disciplines. Recent research advances provide significant opportunities to have a major impact on this devastating disease. This group provides recommendations for both short- and long-term strategies that build on research strengths and opportunities established by the many members of the research community.

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Melanoma is one of the major cancers to affect Caucasian populations. In 2007, an estimated 59,940 Americans will develop cutaneous melanoma, and nearly 8,100 will die of the disease.<sup>1</sup> The National Cancer Institute (NCI) Surveillance, Epidemiology and End Results (SEER) database documents a 619% increase in the annual

incidence of melanoma and a 165% increase in the annual mortality from 1950 to 2000. Mortality has leveled off recently in the United States and Australia in some groups, but it is still generally increasing in other countries such as the United Kingdom, or even in individuals once considered at lower risk due to darker pigmentation, such as Mediterranean populations and California Hispanics. More than 20 years of coordinated clinical and prevention research have helped to identify individuals at high risk, improved diagnosis, enhanced detection of early stages, increased awareness, and developed prevention strategies such as protection from sunlight.

However, knowledge on the molecular mechanism of melanoma progression is still limited, and patients with metastatic disease remain largely incurable. This emphasizes a need to employ cutting edge technologies to design effective therapies. It is increasingly necessary to share and coordinate scientific resources of many groups because the high-tech tools, such as genome- and proteome-wide screening procedures, require large monetary investments and multidisciplinary expertise. This Roadmap highlights the advances, challenges, opportunities, and priorities for

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four major areas in melanoma research and management. As melanoma research advances, our short- and long-term goals will evolve accordingly.

## Basic Research

### Melanoma Classification

Cutaneous melanoma represents a proliferation of activated or genetically altered epidermal melanocytes resulting from complex interactions among genetic, constitutional, and environmental factors. The vast majority of melanomas can be readily distinguished from benign melanocytic nevi by histological examination; however, there are ill-defined subsets of melanomas or atypical pigmented lesions that are notoriously difficult to diagnose. Examples of the latter are melanocytic tumor of unknown malignant potential (MelTUMP), atypical Spitz nevus, and minimal deviation melanoma.<sup>2-5</sup> Such lesions, including some melanocytic nevi simulating melanomas, require additional molecular and other high-tech tools to predict their behavior.<sup>6</sup> This is particularly important since localized disease is largely curable when diagnosed at early stages (melanoma in situ and radial growth phase melanoma [RGP]).

Once melanoma is diagnosed, the currently used histopathologic criteria (depth and level of invasion, ulceration, radial versus vertical growth phase, regression, dermal mitotic activity, tumor infiltrating lymphocyte response, angiolymphatic invasion, satellites) supplemented by sentinel lymph node biopsy (when applicable) can define clinical prognosis and the most optimal surgical treatment. However, they are often not helpful in defining systemic or adjuvant therapy.<sup>7</sup> Thus, we still need better tools for disease subclassification and staging that can provide accurate information on prognosis, clinical management (therapeutic), and discoveries of novel compounds for targeted treatment and prevention.

### Recommendations

- Invest a major effort in the systematic analysis of melanoma lesions for mutation identification, genomic gain and loss (eg, comparative genomic hybridization or high-density single-nucleotide polymorphism array analyses), RNA expression profiling, proteomic and immunologic analyses. These data should be publicly available and used for improvement of diagnosis and prognosis.
- Develop and test hypotheses regarding melanocytic behavior that can be evaluated in the laboratory.
- Use new technologies to form testable hypotheses for new drug discoveries.
- Identify the molecular features of the tumor, its local environment, and host factors that define responsiveness to a wide range of immunologic therapies. Incorporate them into treatment selection.
- Conduct rigorous bioinformatic analyses and functional validation to eliminate functionally irrelevant information (“noise”).

- Identify the most reliable platforms for the above analyses.
- Identify novel molecular or nano-imaging tools to predict behavior of pigmented lesions of difficult diagnosis.

## Melanocyte Development and Transformation

### Melanocyte Development

Major advances have been made recently in understanding the molecular basis of melanocyte development from the neural crest, which includes control of proliferation and differentiation. Animal models to study melanocyte development in vivo include rodents, axolotl, fish, and the frog.<sup>8-16</sup> The differentiation program can be easily assessed by the availability of a wide spectrum of melanocyte-specific molecular tools.

### Recommendations

- Clarify the developmental pathway of melanocytes—from their precursors in the neural crest, through migration and differentiation into melanocytes. This will include testing the involvement of early developmental genes.
- Investigate behavior of melanocyte stem cells in adults, and clarify their relationship with melanoma.
- Create optimal model systems to study human melanocyte development.
- Elucidate functions of the melanogenic apparatus and its product melanin for protection and development of melanoma.
- Explore the comparative biology of melanocytes from distinct tissues (cutaneous, mucosal, and choroidal) and their pathways to melanoma.

### Melanocytes in Culture

Procedures for isolating and growing primary melanocytes in culture from human and other species have been established and pure cultures of melanocytes from a range of pigmentary phenotypes can be analyzed.<sup>17-22</sup> Adult and neonatal melanocytes from different skin types are available commercially or can be established by specialized laboratories. Experimental procedures have been established to examine the differentiation status of the cells employing a wide spectrum of molecular probes.

The real challenge in this area is to define to which degree these pure cultures of proliferating melanocytes are comparable with in vivo conditions.

### Recommendations

- Optimize protocols and establish standardized procedures for melanocyte cultures from different age groups, racial backgrounds, pigmentary phenotypes, genders and body sites.
- Establish a consensus list of melanocyte markers that clearly characterizes the state of differentiation.
- Establish a repository of well-characterized melanocyte cultures in one institute that will be responsible to distribute these cells to different investigators for a nominal fee.

- Promote usage of organotypic skin equivalent or ex vivo skin histoculture as models to mimic the microenvironment of human skin.
- Develop training centers for human skin transplantation into appropriate animal models to study human melanoma genesis or progression in vivo.

### Ultraviolet Irradiation and Melanocyte Transformation

There is evidence from epidemiological observations and genetically altered mice that ultraviolet (UV) exposure can enhance melanoma development; however, the molecular pathway(s) induced by UV radiation (UVR) that lead to malignant transformation are not fully understood. The effect of UVR on melanocytes from different pigmentary phenotypes and the relationship between pigmentation and UVR effects remains to be explored. The factors governing the distribution of melanosomes and their transfer to keratinocytes in response to UVR are unknown, the degree of sun protection needed is not clear, and the effects of sunscreens are difficult to investigate in epidemiologic association studies.<sup>23</sup>

A “divergent pathway” model for the development of melanoma has been suggested. In this model, humans with an inherently low propensity for melanocyte proliferation require chronic sun exposure to drive clonal expansion of transformed epidermal melanocytes. In contrast, among humans with a high propensity for melanocyte proliferation (ie, high nevus counts), exposure to sunlight is a predicted early requirement for carcinogenesis, after which host factors supervene to drive melanoma development. This model is but one potential explanation for the heterogeneity in risk factors (see *Epidemiology and Prevention*).<sup>24-30</sup>

### Recommendations

- Explore the genetic contribution of UV-induced melanoma genesis and the processes by which melanocytes and melanin confer protection from sunlight.
- Study the molecular outcome of UV irradiation in vitro and in vivo.
- Evaluate the role of the stroma in UV-induced melanoma genesis.
- Determine the genetic and epigenetic changes induced by UVA and UVB that contribute to malignant transformation.

## Melanoma Resistance

### Pathways in Apoptosis

Impaired ability to undergo apoptosis provides melanomas with a selective advantage for progression and metastasis and an ability to resist therapy. This emphasizes a need to identify key components of the pathways that can serve as targets for efficient treatment.

Among those identified so far:

- Activation of mitogen-activated protein kinase (MAPK) via constitutively active cell surface receptors or mutational activation of intermediates (such as Ras, BRAF, and c-KIT) in human melanomas elicit constant activation of downstream kinases and the corresponding tran-

scription factor substrates, of which only few have been characterized.<sup>31</sup>

- Altered signaling via phosphoinositide-3 kinase (PI3K), transforming growth factor- $\beta$  (TGF- $\beta$ ), the Janus kinase/signal transducers and activators of transcription (JAK-STAT), and Wnt are implicated in the development of melanomas.
- Changes in the expression and activity of  $\beta$ -catenin are likely mediated by the E3 ligase beta-transducin repeat containing protein ( $\beta$ -TrCP), which is upregulated in tumors such as melanomas. The latter is of further interest since the same family of ligases also affects the stability of inhibitor  $\kappa$ B (I $\kappa$ B), thereby regulating nuclear factor- $\kappa$ B (NF- $\kappa$ B) activity.<sup>32-36</sup>
- NF- $\kappa$ B/Rel, AP1/ATF2, Stat, and p53 transcription factors appear to serve as central regulators of apoptosis.<sup>37-39</sup> Yet, it remains unclear as to which of the NF- $\kappa$ B family members are most crucial for melanoma development. The primary mechanism used to activate them in melanoma and the changes in upstream components of this signaling cascade (TNF receptor-associated factor 2/5 [TRAF2/5], inhibitor kappaB kinase [IKK], TGF- $\kappa$  activated kinase [TAK], TAK-binding protein [TAB], cylindromatosis [CYLD]) are among the topics to study in-depth.

### Recommendations

- Carry out analyses using tumor samples rather than cell lines.
- Analyze the early stages of melanomas when resistance is not fully developed.
- Conduct comprehensive multi-level analyses such as genomic screens or expression profiling at the transcript and protein levels, and post-translational modifications.
- Conduct functional studies to validate results obtained from genomic and proteomic studies.
- Validate findings in appropriate in vivo models of melanoma.

## Tumor Microenvironment

The tumor microenvironment is emerging as a critical factor in tumor etiology, progression, metastasis, and as a target for cancer therapy and prevention. Therefore, there is an immediate need for studies on the contribution of the stroma, immune effectors, and other components of the tumor microenvironment to melanoma growth and metastasis.<sup>40</sup>

### Recommendations

- Explore the interactions of tumor cells and their environment. These include interactions with fibroblasts, extracellular matrix (ECM), endothelium (angiogenesis, intravasation, and extravasation), the lymphatic system (lymphangiogenesis), and inflammatory and immune cells.
- Investigate how tumor and stromal cells can control the local microenvironment.
- Investigate whether “normal” cells of the tumor stroma can be targeted for diagnosis and therapy.

- Investigate how tumor cells escape immune detection and/or destruction.
- Explore the role of the tumor microenvironment with respect to providing the niche for melanoma initiating cells ('melanoma stem cells').
- Use three-dimensional models of human skin and other organs that reflect the microenvironment for melanocytes and melanoma cells and study these in parallel with mouse genetic models.

## Metastasis

### Experimental Correlates to Clinical Melanoma Metastasis

Cutaneous melanomas metastasize locally, regionally to nearby lymph nodes, and systemically to the lungs, liver, soft tissue, brain, and to a lesser degree many other organs. On the other hand, ocular (uveal) melanomas metastasize most frequently to the liver. Different metastasis models offer the opportunity to investigate several steps of the metastatic cascade, the interaction between tumor cells and the surrounding normal tissues and cells, and the molecular basis for homing to specific tissues, mimicking what is seen in patients.

### Recommendations

- Base studies on melanoma metastasis on tissues or cells from orthotopic metastasis models.
- Focus on the interaction in vivo among tumor cells, normal cells, and tissues to determine tissue preference and specificity.
- Investigate molecular and immunological characteristics of cells obtained from experimental metastases from the same and different organs.
- Subject the experimental samples to genomics and proteomics studies. Study corresponding clinical samples to examine molecular leads.
- Exploit imaging techniques to visualize distribution and proliferation of melanoma cells and their interactions with normal host cells.

## Animal Models

### Desired Features in a Melanoma Animal Model

A relevant animal model of human melanoma would help elucidate mechanisms underlying melanoma progression and metastasis and provide an opportunity for better testing of drugs prior to clinical use.

As a surrogate for human melanoma, an animal model should have the following features:

- Resemble human melanoma in its histopathology and molecular pathogenesis.
- Exhibit multistage progression.
- Be highly reproducible with high penetrance.
- Have an intact immune system.
- Exhibit an appropriate response to UVR.
- Be genetically tractable.
- Be cost feasible with respect to tumor latency and breeding/treatment strategies.

- Be translatable to human melanoma, predicting human response in preventive and therapeutic preclinical studies.

### Current Models

A number of animal models of melanoma have been developed over the last two decades.<sup>29</sup> However, none have met the criteria outlined above. For example, the histopathological appearance and graded progression of the melanocytic malignancies arising in animal models have generally been quite distinct from human melanoma, and few model systems have demonstrated appropriate responses to UVR exposure. A number of melanoma-prone transgenic lines of mice have recently been described. These models incorporate inactivation of a variety of tumor suppressors and/or expression of activated oncogenes, validating the role of key pathways implicated in human melanoma genesis. In addition, a subset of these transgenic models has shown a response to UVR that is consistent with that derived from epidemiological studies. Another promising approach has been to induce human melanoma in human skin engrafted onto immunodeficient mice.<sup>22</sup>

### The Use of Animal Models in Preclinical Studies

- To screen for new anti-melanoma drugs.
- To uncover novel molecular targets/pathways associated with melanoma genesis.
- To validate targets and drug-target interactions in melanoma.
- To predict anti-melanoma drug efficacy and safety.
- To assess cellular and serological immune responses.
- To look for tumor-specific biomarkers.

No single model can serve all purposes. Genetically engineered mice can facilitate elucidation of molecular pathways in melanoma, uncover novel targets of drugs, and verify that they actually hit those targets.

### Recommendations

- Develop an extensive repository consisting of various models of human melanoma. This repository should include an exhaustive representation of various human and mouse melanoma cell lines and tissues, as well as the actual mouse melanoma models themselves; these should be rigorously validated for their relevance to human melanoma.
- Establish a mechanism or program that would permit integrated preclinical tests using human melanoma models. Initially these models could be used for validating molecular targets, assessing the efficacy of candidate drugs and drug-target interactions, and perhaps screening anti-melanoma agents.

## Immune Responses

### Present Status

The occasional spontaneous regression of primary melanomas or interleukin-2 (IL-2)-induced complete regression of metastatic melanoma has inspired intensive immunological investigations.<sup>41,42</sup> Molecularly defined melanoma antigens

have been identified and the goal is to harness the host immune system to control the tumor.

Initial immunotherapeutic interventions focused on development of monoclonal antibodies (MAbs) and cytokines designed to expand the cellular immune responses against tumors. Due to funding and patent issues, MAb development has not progressed in the melanoma field (although MAbs have become quite important in the treatment of other malignancies). Instead, the melanoma field has focused on immunologic therapies based on tumor-reactive T cells. Recently, it has become possible to treat melanoma patients with *in vitro*-expanded, autologous T lymphocytes with reactivity against cultured melanoma cells.<sup>41</sup> Clinical responses have been seen, including long-term complete remissions. However, the *in vitro*-expanded T cells are often rapidly eliminated *in vivo*. Studies aimed at prolonging the survival of transferred T cells are in progress.

Efforts continue to develop melanoma vaccines. Past efforts using inactivated pooled allogeneic melanoma cells have been unsuccessful in randomized trials, although it is difficult to know how immunogenic these vaccines were. There also has been an extensive effort to vaccinate against specific proteins on melanoma cells using peptides alone, with adjuvant, or presented by dendritic cells. These approaches appear to be more immunogenic, although there remain few clinical responses.<sup>42-46</sup>

More recently, we have learned about mechanisms in place to limit the immune response to self antigens. Molecules on T cells involved in turning off the immune response, such as CTLA4 and PD-1, can be blocked (by MAb or small molecules). This appears to be a potent mechanism to unleash T-cell responses against self antigens. The other major mechanism of T-cell regulation is through regulatory T cells ( $T_{reg}$ ). These cells express several surface markers, including CD4/CD25, GITR, CTLA4, and Neuropilin, and the intracellular transcription factor FoxP3, which is their most selective marker.<sup>47-52</sup> Antibodies can be used to target the cell surface markers, and FoxP3 should be an excellent target for drug discovery.

Newer methods of immunization are being explored using DNA vaccines and MAbs that block inhibitory T-cell signals (eg, anti-CTLA4 MAb). With the advent of these new approaches, and recent technical innovations that have led to T-cell function assays with high sensitivity, vaccine strategies may find a resurgence warranting randomized trials. However, the major challenge to the cancer vaccine field remains to demonstrate that these T-cell effects have clinical relevance.

### Recommendations

- Develop a MAb program in melanoma.
- Develop clinical trial designs capable of evaluating anti-tumor effects so that melanoma antigens can be identified that induce an efficient tumor-destructive immune response.
- Establish which costimulatory signals, in the presence of a source of tumor antigens, most effectively facilitate the

generation and expansion of a tumor-destructive immune response.

- Identify the downregulatory mechanisms that play pivotal roles in inhibiting immunity to melanoma antigens and identify how to best overcome this regulation using biological agents such as monoclonal antibodies and/or small molecule drugs.
- Establish the individual susceptibility of the host to mount effective immune responses.
- Establish the individual susceptibility of the tumor to be controlled by the immune system and attempt to find ways to overcome the problem that melanomas often lose epitopes selected as therapeutic targets and/or the ability to present such epitopes by major histocompatibility complex (MHC) class I and II molecules.
- Develop new methods for immune monitoring that accurately reflect the immune stimulatory potency as well as the tumor-inhibiting effects of the vaccine and correlate the results with clinical outcome.
- Investigate methods to suppress  $T_{reg}$  number and function. For example, administration of cyclophosphamide can eliminate  $T_{reg}$  cells with therapeutic benefit in some animal models.
- Establish improved genetic mouse models for immunological studies, including vaccines and combinations of immunotherapies.

## Serum Diagnostics

### Blood Screening for Cells, Proteins, and Nucleic Acids

Analysis of peripheral blood has the potential to significantly impact clinical care of melanoma and to improve our understanding of metastatic disease.

### Recommendations

- Assess the significance of melanoma cells in peripheral blood and/or bone marrow.
- Establish serum proteomics to determine if alterations in serum proteins can be used to either detect various types of cancer or to determine tumor cell death during treatment.
- Develop procedures for serum nucleic acid measurements. Analysis of these molecules can lead to the development of new markers such as DNA methylation and allelic imbalance of genes relevant to melanoma.

## Clinical Research

### Overview

To date, no therapy improves the overall survival of patients with stage IV melanoma. The median survival for patients with stage IV melanoma is approximately 9 months and 5 year survival rates are 5% to 15% depending upon sites of metastatic disease. In addition, there is no generally accepted standard care for patients with metastatic melanoma. The range of options includes observation, surgical resections of limited metastatic disease, palliative resections for local con-

trol, therapy with dacarbazine or temozolomide, combination chemotherapy, or immunotherapy.

The feasibility of reducing melanoma recurrence and mortality with adjuvant therapy has been investigated in more than 100 randomized controlled trials. Past efforts have focused on interferon  $\alpha$ 2b (IFN), vaccines, or both. High-dose interferon is approved by the US Food and Drug Administration for treating patients with primary melanoma lesions thicker than 4 mm or with regional lymph node involvement. However, high-dose IFN is used inconsistently in the United States because of its considerable toxic effects and modest benefits on relapse-free survival without a reproducible benefit in overall survival. Past trials of vaccines composed of autologous or allogeneic melanoma, cell lysates, and peptides have been negative, probably due to their minimal ability to induce immune responses against relevant tumor antigens. One way around this has been to use adoptive immunotherapy and antibody-mediated therapies to generate massive numbers of activated T cells or MAbs *in vitro* that can be infused into patients.

### New Strategies for Characterization, Monitoring, and Therapy

Until recently, the most significant difficulty in developing new therapies for melanoma has been the lack of potential targets and agents. Now, some of the molecular pathways relevant to melanoma biology have been described. Constitutive activation of the tyrosine kinase (TK) Ras/Raf/MAPK pathway is a frequent and early event in melanoma development. The challenge is to develop agents that target these aberrant molecular pathways. An example of such an agent is sorafenib (BAY 43-9006), an oral TK inhibitor with a wide spectrum of targets (v-raf murine sarcoma viral oncogene homolog [RAF], vascular endothelial growth factor-1 [VEGFR1], platelet-derived growth factor [PDGFR], Flt3, and c-KIT) that inhibit the MAPK cascade and pro-angiogenic pathways.<sup>53</sup> As a single agent, antitumor activity with sorafenib has been difficult to demonstrate. Sorafenib is already in clinical trials in combination with chemotherapy, which represents the first attempt to combine TK blockade with cytotoxic chemotherapy in melanoma. Newer, more potent and selective inhibitors of BRAF, MAP kinase kinase or Erk kinase [MEK], and other TKs are entering clinical trials and represent an exciting new approach to melanoma treatment.

Knowledge of the molecular biology of melanoma should be used to identify existing agents that target biologic pathways critical to the melanoma phenotype. These agents, used either alone or in combination with standard therapy, have the potential to be more selective for melanoma and less toxic to the patient. Clinical trials need to utilize agents with well-understood mechanisms of action that interact with pathways critical for melanoma pathogenesis. Molecular characteristics of tumors, including expression of the target, need to be assessed as part of clinical trials. The acquisition of tumor and nontumor tissue before, during, and after therapy is critical for measuring the effect of treatment and defining predictive features of outcome. The analysis of tumor and host

tissues for evidence of response to therapy will require developing methods for the optimal use and evaluation of small tissue samples obtained in the course of therapy.

### Establish Tumor/Tissue Banks and Identify Biomarkers

Key biomarkers and surrogate end points need to be established for epidemiological studies and prevention or therapy trials. The development of biomarkers and surrogate end points will require a series of tissue banks—including normal skin, melanocytic lesions, melanoma, and serum banks linked to complete clinical data. Melanoma research in particular has been hampered by limited access to large numbers of melanoma tissues with associated clinical information that will permit the development of biomarkers. Well-annotated tumor tissue arrays with proper bioinformatic tools can enhance marker discovery and validation. This would require the establishment of optimal methods of procurement, preservation, and analysis, the development of a prioritized distribution process, the design of flexible consent forms that maximize tissue availability, and protect subjects' identity. The NCI has been instrumental in supporting an effort of multi-institutional melanoma researchers for developing diagnostic and prognostic tissue arrays. These tools are an important resource to validate biomarkers that are currently discovered in genome-wide screens. It is important to recognize that in melanoma, there are a number of unique challenges to the collection of primary melanoma tissue for biorepositories and for research purposes. The majority of primary melanomas are diagnosed in the community, often in a dermatologist's or primary care provider's office, making the collection of the tissue difficult. In addition, the majority of primary melanomas are thin, with most of the lesion needed to establish the histological diagnosis and for microstaging of the lesion. Finally, there are medical/legal and privacy protection concerns that are further impediments to the collection of primary melanomas. These issues have significantly limited access to primary melanomas and must be addressed in a comprehensive way in order to advance the melanoma field.

The discovery of molecular markers that identify aberrant pathways in melanoma should facilitate the detection of occult disease and aggressive behavior as well as the selection of patients for specific therapy. These markers also can be used to monitor the effectiveness of therapy for individual patients. Consequently, smaller, more targeted clinical trials could be performed, which seek bigger differences in outcome.

### Diagnostic and Imaging Techniques

Many therapeutic interventions produce physiologic, molecular, immunologic, or biochemical effects that could be measured non-invasively or with minimally invasive techniques to help monitor clinical benefit early during treatment.

The analysis of tumor and host tissues for evidence of efficient response to therapy will require:

- Minimally invasive surgical and nonsurgical techniques for obtaining tumor tissue serially.

- Methods for the optimal use and evaluation of small amounts of fresh, frozen, and paraffin-fixed tissue samples obtained in the course of therapy.
- Improved imaging techniques (positron emission tomography and magnetic resonance imaging augmented with specific probes) that will provide functional (eg, anti-angiogenesis, immune-mediated mechanisms), molecular (eg, apoptosis, inhibition of specific signaling pathways), and nano-imaging data sufficient to determine the effect of the particular therapy on its putative target and guide minimally invasive biopsies to the regions of maximal interest.
- Immune monitoring assays that will serve as accurate measures of potency and surrogate markers for efficacy.
- Development of multicenter infrastructures to validate biomarkers for melanoma diagnosis, prognosis, and therapy outcome prediction.
- Reduction of obstacles for the collection of primary melanomas.

### Clinical Research Infrastructure

There is a growing consensus regarding the importance of streamlining and modifying current clinical trials systems. We look forward to collaborating with the Clinical Trials Working Group, (established by the NCI) to improve the clinical trials system and enhance access to therapeutic agents. Of particular relevance are the continued efforts to optimize clinical trial design and explore alternative trial designs, which address the activity of new strategies such as inhibiting the growth of tumor cells as opposed to killing them. The NCI-sponsored cancer Biomedical Informatics Grid (caBIG) is an important new initiative that can greatly facilitate our ability to accomplish the outlined goals.

Continuing to forge academic/industry/NCI partnerships is critical for drug development. Both industry and academia are developing new therapeutic agents; however, the broader scientific community often does not have access to these agents for preclinical and clinical studies. Furthermore, many of these agents are not evaluated for treating melanoma. In addition, proprietary concerns also limit the use of novel agents in combination, especially when multiple pharmaceutical companies are involved. The development process could be facilitated by broad master agreements among the NCI, pharmaceutical industry, and academia that assure the research community's access to these investigational agents while protecting the interests of all parties.

### Development of the Melanoma Working Group

To accomplish the goals and make therapeutic progress, we propose the creation of a Melanoma Working Group, which would act as a centralized resource to assist in the coordination of cross-discipline biology/translational/clinical studies in melanoma research. In particular, there is a growing awareness of the widening gap between extraordinary scientific discoveries and the ability to apply this knowledge in the clinical arena. There is a critical need to bridge this gap and to coordinate and prioritize laboratory and clinical studies. This working group would include leadership representatives

from the Special Programs of Research Excellence (SPORE) Cooperative Groups including the American College of Surgeons Oncology Group (given the critical role of surgeons in melanoma treatment), Cancer Centers, NCI, Cancer Prevention, Dermatology, and Pathology. We envision the Melanoma Working Group as having a role distinct from single institution SPOREs and Program Projects occurring at institutions without SPORE grants. This working group could facilitate collaborative research efforts across institutions and disciplines and play an active role in prioritizing research grants. It could advise the NCI on important new areas of investigation in melanoma and on emerging technologies, new funding opportunities, etc. The establishment of the Melanoma Working Group should be cost effective. The Melanoma Working Group should foster international links with the leading cooperative melanoma groups such as the European Organization for Research and Treatment of Cancer (EORTC) Melanoma Group and the Melanoma Genetics Consortium (GenoMEL), and envision international intergroup projects, a necessity in any relatively rare disease. Communication should also be facilitated through the newly established Society for Melanoma Research.

### Recommendations

- Generate preclinical data that identify and validate appropriate targets in melanoma and make the data available to researchers.
- Create Tumor Tissue Banks with dedicated funding to enable the acquisition of precursor melanocytic lesions, primary melanomas, and pre- and post-treatment patient tumor specimens to conduct basic and clinical research. Establish guidelines for optimal methods for procurement, preservation, analysis, and distribution. These tumor banks will provide unique opportunities to study early stages of this disease and develop biomarkers.
- Establish Drug Banks composed of new and developing agents that may affect melanoma and make these Banks available to basic and clinical researchers.
- Validate newly discovered biomarkers from genetic, genomic, and proteomic analyses.
- Develop analyses of gene function and pathway dissection most critical for melanoma cell killing and/or stasis in a tissue environment.
- Develop in vitro screening models for new drugs that take into account the complex tissue environment of tumor cells.
- Participate in Rapid Access to Intervention Development (RAID) and other NCI funding mechanisms to more rapidly bridge the gap between the basic research findings and clinical trials.
- Validate animal models suitable and relevant for therapy studies.
- Establish a centralized mechanism for production of patient specific therapies, especially cellular therapies.
- Repeat vaccine studies in the presence of inhibitors of immune regulation.

- Establish the Melanoma Working Group with an international platform.

## Epidemiology and Prevention

### General Population Studies

#### Risk Factors

The following risk factors for melanoma have been established:

- Individual phenotype, such as fair skin that tans poorly, light-colored eyes, red or light-colored hair, freckling, and high nevus counts and presence of dysplastic nevi.
- Exposure to UVR, in particular, high levels of intermittent sun exposure. In this respect the role of tanning in tanning parlors and UVA in melanoma development should be investigated. Sun exposure seems to increase the development of nevi and dysplastic nevi among predisposed individuals.
- Family history of melanoma.

#### Prevention of Melanoma

Primary prevention has centered on using sunscreens and avoiding exposure to intense intermittent UVR. However, the complexity of the relationship between sun exposure and risk, the long latency for the development of melanoma and its relatively low incidence have hampered attempts at evaluating the factors that prevent its development and progression. Limitations in the ability to retrospectively measure sun exposure further hampers epidemiological studies of UVR exposure and melanoma risk. Furthermore, controversy remains about the efficacy of these interventions to decrease melanoma incidence and mortality, with some studies suggesting that sunscreen use is not effective as a melanoma prevention strategy in all situations.

Secondary prevention, or screening for early-stage melanoma, has not yet been rigorously evaluated but holds promise for reducing mortality. The Australian experience demonstrates that public awareness of melanoma and its risk factors has led to a flattening in mortality trends and a decline in incidence rates among younger age groups. However, even in Australia where the most progress seems to have been made, older people—older men in particular—are continuing to present with deep melanomas, which account for a large proportion of the mortality from melanoma. Furthermore, the interventions used in Australia have not been implemented in other parts of the world and may not be appropriate in more temperate climates.

#### Familial Studies

So far, family studies have identified four high-risk susceptibility genes for melanoma. Inherited mutations in *CDKN2A*, which codes for both the cyclin-dependent kinase (CDK) inhibitor p16INK4A and the tumor-suppressor P14ARF, have been shown to confer susceptibility in around 40% of families with three or more cases of melanoma, while mutation frequency in the families with only two cases of melanoma is much lower, except in certain geographic regions in

which there is a common founder mutation. Mutations in *CDK4*, which confer resistance to inhibition of melanomagenesis by p16INK4A also are causal but very rare. On the other hand, mutated or deleted p14ARF also increases risk of melanoma. Finally, a susceptibility locus has been mapped to chromosome 1p22, but the gene(s) associated with this locus have not yet been determined. Thus, novel methods have to be applied to determine other melanoma susceptibility genes in melanoma-prone families, which is the mission of GenoMEL, the melanoma genetics consortium ([www.genomel.org](http://www.genomel.org)).

### Molecular Epidemiology Studies

The interactions between low-penetrance genes and non-genetic risk factors (eg, UVR exposure) are likely to contribute to melanoma etiology. Genes that reduce eumelanin production in skin, hair and eyes can be considered susceptibility genes. A good example is the effect of inherited variants in *MC1R*. These variants increase the proportion of melanocyte pheomelanin (at least in the hair follicle), and inheritance of these variants is predictive of red hair and freckles. These variants also appear to act as low risk melanoma susceptibility alleles even in people without red hair. Thus, *MC1R* variants may provide information about melanoma risk beyond that predicted by pigmentation and cutaneous phenotype alone.

The finding of activating *BRAF* and *RAS* mutations in a high percentage of melanomas is now being investigated in relationship to UVR in combination with genotypic and phenotypic susceptibility. Elucidation of the relationships between somatic mutations, genetic susceptibility, and epidemiological factors is likely to help define distinct genetic pathways that lead to melanoma.

### Genetic Studies in Families

We need to better understand the mutation penetrance in melanoma susceptibility genes and the risk of cancers other than melanoma in families that carry these mutations. We need to understand how these susceptibility genes correlate with the abnormal nevus phenotype (which is the most potent phenotypic risk factor for melanoma) and how they interact with sun exposure to cause melanoma.

Genome-wide screens for new melanoma susceptibility genes are needed, using both association studies (for low-penetrance genes) and multiplex families (for high-penetrance genes). Similarly, genome-wide screens for traits related to melanoma risk, such as propensity to develop nevi, are essential, because some of the genes underlying these traits also may be melanoma susceptibility loci. An extension of this work would be to conduct targeted SNP genotyping of a panel of low-penetrance candidate genes. Ideally, agreement should be achieved prospectively to screen candidate genes in concurrent large sample sets such as those collected by consortia. Large case-control studies can be pooled for analysis (including test and validation sets), as well as cohort studies designed specifically to identify genes governing prognosis and outcome.

## Challenges, Opportunities, and Priorities

Risk-prediction and risk-reducing interventions in high-risk populations will be possible only when the presence of consistent, replicated associations between genes and melanoma is established. There is a need to have large-scale population-based studies and randomized trials on surrogate targets (eg, nevi) to clarify the role of these genes in melanoma etiology, as well as to better understand the utility of sunscreens and other sun blockers (ie, specific clothing) in the prevention of melanoma. The suggestion has been made that chronic sun exposure might even be protective for melanoma and, therefore, the complex relationship between the sun and melanoma risk must be better understood.

Effective intervention by practitioners and public health experts is a major challenge that could be possible only when we have a clear understanding of the biology of melanoma. We need to determine if multiple pathways to melanoma development exist, and if the risk factors for each pathway are unique. The factors associated with indolent and aggressive melanoma have to be identified to improve diagnosis of melanoma and to predict outcome.

Data collection in family studies and the establishment of a collaborative consortium with centralized databases is critical. Pooling of data from multiple groups worldwide will allow assessment of the effects of sun exposure and phenotypic factors on penetrance of mutations in the high-penetrance melanoma genes identified to date and to identify new genes. Additionally, these collaborative studies will enable more accurate estimates of the risk of other cancers in mutation carriers.

The identification of low risk genes and the roles of genetic and environmental factors in melanoma etiology and progression remain among the greatest challenges that require the development of collaborative approaches and the use of novel methodologies. This includes collaboration among existing case-control studies of melanoma, such as the Genetic Epidemiology of Melanoma (GEM) and GenoMEL studies, large cohorts, such as the NCI-sponsored cohort consortium, and the development of new large case-control and/or cohort studies. Collaborative efforts can develop a robust understanding of the complex measures important to the study of melanoma, such as sun exposure and phenotype, and will provide sufficient statistical power to detect the effects of low penetrance genes and interactions of these genes with exposures.

### Recommendations

- Assess current study designs and develop and evaluate new study designs.
- Develop ways to efficiently increase the size/power of epidemiological studies over multiple geographic locations and study populations to investigate the etiology of melanoma.
- Devise and use a standardized set of questionnaires and data ascertainment procedures to assist in better comparing or merging data from various studies.
- Develop methods to evaluate different UVR exposure measures, such as ambient estimated UVR-erythemal

values, self-reported measures of hours of exposure over a lifetime versus more qualitative measures of UVR exposures, and tanning parlor UVR exposures over time.

- Develop a method to prioritize scarce resources for genome-wide analyses versus candidate gene association studies. To assess the inter-study reliability of genome-wide and candidate gene analyses.
- Initiate epidemiological studies of progression and prognosis in families as well as population-based case series. Determine whether there are constitutively indolent lesions and constitutively aggressive lesions, and whether disease progression is modified by the behavior of the individual, genetic predisposition, or the characteristics of the tissue surrounding the lesion.
- Develop effective, well-evaluated, educational programs for physicians and the public to increase awareness of the characteristics of melanoma. Support grass root efforts by advocate groups for prevention studies.
- Implement and evaluate large community-wide melanoma prevention programs based on the Australian model. Evaluation of such a model needs to rely on mortality or the incidence of thick lesions, not just overall melanoma incidence, as an end point.

## Training

### Overview

There is a need to encourage trained physician-scientists to collaborate with basic and other scientists, as well as clinicians in melanoma research. Funding by the NCI (potentially in partnership with other sister National Institutes of Health institutes, such as the National Institute of Arthritis and Musculoskeletal and Skin Diseases [NIAMS]) could help support a year of basic or clinical research training in this field. In addition, career development awards and training grants specific to melanoma at the young investigator level will encourage talented individuals to focus on this disease.

Within the past 5 years, several major centers have established dedicated Melanoma Programs or Centers of Excellence, which include clinical and basic research departments and cut across multiple relevant disciplines. These programs investigate melanoma and skin cancer as a unique discipline. They provide a formal forum for melanoma researchers and clinicians, as well as resources and opportunities for specific training in melanoma.

### Long-Term Objectives

#### Goals

- To recruit and train junior physicians, graduate students, and postdoctoral fellows in the field of melanoma research.
- To establish a mentorship program to educate the basic molecular and cellular principles of melanoma biology.
- To secure funding for training grants and fellowships specific for melanoma.
- To identify career pathways for trainees.

## Recruitment

At this time, melanoma-specific recruitment is active in the three existing centers of SPORE in Skin Cancer and foundations such as the Melanoma Research Foundation (MRF) solicitations. Applications for T32 pre- and post-doctoral training grants involving leaders in the field from different institutions specifically for training in melanoma can lead directly to enhanced recruitment.

## Mentorship Program

Junior scientists, students, and faculty-in-training are instructed in the principles and issues of clinical melanoma and basic science studies of human melanoma. The detailed areas to be taught include melanoma immunology and bio-immunotherapy; melanoma molecular biology; biochemistry unique to melanin production and response to UVR and inflammation in epidermal cells; human genetics; and other areas that become important for successful research on melanoma. The mentorship program will include both basic and clinical investigators. This Career Development Program will emphasize specific high-priority research areas.

## Solicit Funding for Melanoma-Specific Training and Career Opportunities

Currently, the NIH and Department of Defense support several cancer site-specific pre- and postdoctoral training programs, but none exists for melanoma.

## Short-Term Objectives

- To embrace non-melanoma researchers who are in current funded and nonfunded programs as part of the plan to expand our current research base.
- To create an infrastructure to enhance the ability of young investigators to compete effectively for K08, R21, and R01 grants.
- To increase the membership of the Society for Melanoma Research by recruiting of dermatologists, dermatopathologists, and clinical immunologists.
- To prepare series of seminars and educational materials on melanoma.

In summary, training at all levels is needed in the melanoma research area. Communication and visibility may initially attain the major means of recruiting and expanding our base. Future training is dependent on funding of trainees including the approval of melanoma-specific training grants.

## Appendix

### Participating members of the NCI Workshop "A Strategic Action Plan for Melanoma Research, February 2007," Bethesda, MD:

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