

Roadmap for New Opportunities in Melanoma Research

Society for Melanoma Research (SMR)
and
Melanoma Research Foundation (MRF)

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Preface

Melanoma is one of the major cancers to affect Caucasian populations. In 2004, an estimated 55,000 Americans developed cutaneous melanoma; nearly 8,000 will die from the disease. The NCI 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 USA and Australia in some groups, but it is still generally increasing in other countries such as the UK. More than 20 years of coordinated clinical and prevention research have likely reduced mortality, helped to identify individuals at high risk, improved diagnosis, improved detection of early stages, increased awareness, and developed prevention strategies such as protection from sunlight.

However, the etiology of melanoma remains poorly understood, and the median life span of patients with advanced disease is less than a year because there are no effective therapies once the tumor has spread to vital organs. Therefore, there is an urgent need to employ cutting edge technologies to help identify melanoma-specific targets and further delineate the melanoma-immune system interplay in order to design more effective therapy. It is increasingly necessary to share and coordinate scientific resources of many groups because the rapid introduction of high-tech tools, such as genome- and proteome-wide screening procedures requires large monetary investments and multi-disciplinary expertise. This Roadmap highlights the advances, challenges, opportunities, and priorities for four major areas in melanoma research and management. But this document is by no means an all-inclusive review of the field; rather, it is meant to stress the most obvious areas that require current attention. As melanoma research advances, our short- and long-term goals will evolve accordingly.

A. Basic Research

I. Melanoma Classification

The presence of a melanocytic skin lesion that changes color, size and contours can be detected by routine skin examination. However, the distinction between benign and malignant lesions is often clinically and histologically ambiguous, because benign melanocytic tumors include melanocytic nevi (junctional, compound, intradermal), and their variants (congenital nevi, Spitz nevi, and dysplastic nevi, among others). In addition there is a group of lesions termed melanocytic tumor of uncertain malignant potential or atypical melanocytic proliferation for which the malignant state is questionable. Once diagnosed, the criteria for prognosis of stages I and II melanomas are suboptimal, because they are based only on depth and level of invasion and ulceration.

Based on current criteria, it is possible to provide only partially meaningful and predictive prognostic information. We need better tools for disease subclassification and patient staging that can provide accurate information for: prognosis for recurrence; clinical management (ie, therapeutic decision-making); 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, CGH or high density SNP array analyses), RNA expression profiling, proteomic and immunologic analyses.
- Make the data publicly available.
- Use the information gained from these screens to enhance classification and improve criteria for diagnosis and prognosis.
- Use the information to generate new bases for classification and to develop and test hypotheses regarding behavior that can be evaluated in the laboratory.
- Use data derived from new technologies to form testable hypotheses for new drug discoveries.
- Identify the molecular features that define responsiveness to current immunologic therapies and underlying host differences that affect the outcome of these therapies. Incorporate them into treatment selection.
- Conduct rigorous bioinformatic analyses and functional validation to separate the biologically critical data from the large amount of functionally irrelevant information (“noise”).
- Identify the most reliable platforms for these analyses (especially quantitative ones) as well as the significant contributions of stroma or other “contaminating” cell populations within the analyses.

II. Melanocyte Development and Transformation

Melanocytes are the pigment-producing cells that provide protection against the sun’s harmful ultraviolet (UV) radiation. They are also the precursor cells to the devastating skin cancer, melanoma. It is very important to understand the basics of melanocyte development and the impact of UV light on these cells. This information is required to characterize distinct steps in melanoma genesis and progression, to understand molecular mechanisms involved in this process, and to develop novel therapeutic options.

Melanocyte development

Major advances have been made over the past few years in understanding the molecular basis of melanocyte development and differentiation from the neural crest, the transcription factors that govern their development, the genes they control, the pathways leading to melanin synthesis, and the general similarities and differences in the control of proliferation and pigmentation of melanocytes from human, murine and other sources. Additionally, several model systems to study melanocyte development *in vivo* are available, including mouse, axolotl, fish (e.g., *Xiphophorus*), and frog (e.g., *Xenopus*) models. The differentiation status of melanocytes can be easily assessed by the availability of a wide spectrum of antibodies that recognize melanocyte-specific proteins.

Recommendations

- Investigate whether melanocyte precursor (stem) cells exist in adults, and if so, identify their location, nature, and relationship to melanoma.
- Create a model system to study human melanocyte development, as the biology of mouse melanocytes differs in several aspects.
- Elucidate the function of different melanin types and their contributions to melanoma. Also, clarify the mechanisms by which melanosomes are distributed and transferred to keratinocytes.
- Conduct intensive basic research to clarify the developmental pathway of melanocytes—from their precursors in the neural crest, through migration and differentiation into melanocytes.
- Explore the differential biology of melanocytes from distinct tissues (cutaneous, mucosal, choroidal) and the distinct changes that lead to melanomas.
- Assess the involvement of early developmental genes in melanocyte genesis using mouse mutants and genetically modified mice. Evaluate the contribution of melanocyte stem cells and melanoblasts to melanoma.

Melanocytes in culture

Procedures for isolating and growing primary melanocytes in culture from human, mouse and other species have been established in several laboratories. Pure cultures of melanocytes from different species and from a range of pigmentary phenotypes can be analyzed. Experimental procedures have been established to examine the differentiation status of the cells employing the wide spectrum of antibodies that recognize melanocyte-specific proteins.

Pure cultures of normal melanocytes are needed to carefully analyze changes in tumor cells. Currently, the most common source for human melanocytes is newborn foreskins because the tissues can be easily obtained and the cells are highly proliferating. However, these melanocytes are obtained exclusively from young males, which may bias the results when comparing them to melanoma cells that originate from adults. Adult melanocytes from discarded tissues are needed for routine studies. In addition, immortalized human melanocyte lines that are similar to normal melanocytes can alleviate some of the need for repeated culturing of melanocytes with a finite life span. Established procedure for co-culturing melanocytes with keratinocytes including organotypic cultures are also needed to simulate the skin environment.

Recommendations

- Optimize protocols and establish standardized procedures for melanocyte isolation and culture conditions to be used by all laboratories.

- Establish a consensus list of melanocyte markers that clearly characterizes the state of differentiation.
- Widen the sources of melanocytes to include adult skin from different age groups, racial backgrounds, pigmentary phenotypes, and genders.
- 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.
- Attempt to immortalize melanocytes without affecting major characteristics, such as differentiation and malignant potential to generate melanocytic cell lines needed by the scientific community.
- Develop training centers for organotypic cultures as models to mimic the microenvironment of human skin.

UV irradiation and melanocyte transformation

UV light and processes induced by UV have been explored for several years, providing critical insight into the effects on pigmentation, gene expression, and proliferative responses. There is evidence from epidemiological observations and genetically altered mice that UV exposure can enhance melanoma development; however, the molecular changes induced by UVA and UVB irradiation that lead to malignant transformation are not well known and the various steps by which melanomas develop after UV irradiation are not fully understood. The effect of UV irradiation on melanocytes from different pigmentary phenotypes and the relationship between pigmentation and UV effects remained to be explored. More information is required regarding the interaction between different types of melanin and UV irradiation. The factors governing the distribution of melanosomes and their transfer to keratinocytes in response to UV light are unknown, the usefulness of sun protection is not clear, and the negative effects of sunscreens are unexplored.

Much of the uncertainty surrounding sunlight's role in causing melanoma stems from the assumption that all cutaneous melanomas arise through the same pathogenic pathway, but there is no *a priori* reason why this should be so. 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 C. Epidemiology and Prevention*).

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 irradiation, which contribute to malignant transformation.

III. Melanoma Resistance to Genotoxic Stimuli

Pathways in apoptosis

Impaired ability to undergo programmed cell death provides melanomas with a selective advantage for progression and metastasis and a notorious ability to resist therapy. Among the key contributors to melanoma development, progression and resistance to apoptosis are constitutively active signal transducers and corresponding transcription factor effectors. Yet, despite extensive studies, there is still an urgent need to identify new pathways and key components that can serve as targets for efficient treatment.

Among those identified so far:

- Activation of MAPK via constitutively active cell surface receptors or mutational activation of intermediates (such as Ras and BRAF) in human melanomas elicit constant activation of the corresponding transcription factors substrates, of which only few have been characterized. Thus, efforts are required to identify regulatory components that are affected by MAPK signaling and which directly contribute to the progression of melanomas.
- Signaling via PTEN, TGF- β , JAK-STAT and WNT are implicated in the development of melanomas.
- Changes in the stability of key regulatory components in melanomas are also critical in conferring resistance to apoptosis. For example, changes in the expression and activity of β -catenin are likely mediated by the E3 ligase β -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 I κ B, thereby regulating NF- κ B activity.
- Four families of transcription factors (NF- κ B/Rel, AP1/ATF2, Stat, and p53) appear to serve as central regulators of apoptosis. Yet, it remains unclear which of the NF- κ 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 (IKK, TAK, TAB, 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 screen, 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 models of melanoma.

IV. 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. Basic melanoma research has been mainly concentrated on elucidating the molecular changes associated within the tumor itself with less emphasis on the contribution of the tumor microenvironment on invasion, angiogenesis, and metastasis. Therefore, there is an immediate need to perform studies that will enhance our understanding of the contribution of the stroma, immune effectors, and other components of the tumor microenvironment to melanoma growth and metastasis.

Recommendations

- Explore the interactions of tumor cells and their environment. These include interactions with fibroblasts, extracellular matrix (ECM), endothelium (angiogenesis, intravasation, and extravasation), lymphatic system (lymphangiogenesis) and inflammatory and immune cells.
- Investigate how tumor cells control the microenvironment by producing proteolytic enzymes, for example, and by growth factors of normal cells infiltrating the tumor.
- Investigate whether and how normal cells in the tumor stroma can be targeted for diagnosis, prognosis, and therapy.
- Investigate how tumor cells escape immune detection and/or destruction.
- Develop 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.

V. Metastasis

Experimental correlates to clinical melanoma metastasis

Cutaneous melanomas metastasize locally, regionally to nearby lymph nodes, and systemically to the lungs, liver, and brain (and sometimes to the intestines). On the other hand, ocular (uveal) melanomas metastasize most frequently to the liver. Only limited experimental models reproduce some patterns of spreading. Metastasis models can provide an unlimited number of cells for continuous study. 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 of the formation and biology of 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 the distribution and proliferation of melanoma cells, to follow the kinetics of spread, and to learn their interactions with normal host cells.

VI. 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 by providing ample pathological samples and an experimental system to test hypotheses derived from human clinical and epidemiological data. It will also provide an opportunity for better preclinical testing of drugs prior to clinical use.

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

- Resemble human melanoma in its histopathology and molecular pathogenesis.
- Exhibit multistage progression, from nevus-like lesions to metastases.

- Be highly reproducible with high penetrance.
- Have an intact immune system.
- Exhibit an appropriate response to ultraviolet radiation.
- Be genetically tractable.
- Be cost feasible with respect to tumor latency and breeding/treatment strategy.
- 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. Early animal melanoma models include the freshwater fish *Xiphophorus*, the opossum *Monodelphis domestica*, and a number of wild-type rodent systems. 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 UV exposure. The advent of genetic engineering and other recent technological advances have catapulted the mouse to the forefront of human cancer modeling. A number of melanoma-prone transgenic lines of mice have recently been described, providing critical insights into genetic mechanisms that predispose melanocytes to malignant transformation. 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 UV radiation that is consistent with that derived from epidemiological studies. Another promising approach has been to induce human melanoma through UV irradiation of immunodeficient mice engrafted with human newborn foreskin. The creation of this current crop of melanoma models represents significant progress in the field; however, much still remains to be accomplished.

Ways to improve melanoma models

Although mouse models with melanoma progression have been developed, early melanocytic lesions are not a good match with benign and dysplastic nevi in the human population. Moreover, metastatic disease is still relatively rare in mouse models. The latency associated with the development of primary melanoma and their metastases is generally too long to make the currently available models useful for preclinical studies (see below). More rigorous control of the molecular/genetic events implicated in the development of human melanoma must be asserted in the mouse. Fortunately, recent breakthrough technology now permits impressive control of the activation or inactivation of gene function in both time and space. For example, it is now feasible to “knockout” a designated tumor suppressor gene or “knockdown” its expression specifically in premalignant melanocytes. This technology will ultimately allow scientists to develop mouse models that are a more accurate reflection of human melanoma, and to determine which genes are involved in which stages of melanoma development.

The use of animal models in preclinical studies

Animal models of human melanoma could be used in a preclinical setting to:

- Screen for new anti-melanoma drugs.
- Uncover novel molecular targets/pathways associated with melanoma genesis.
- Validate targets and drug-target interactions in melanoma.
- Predict anti-melanoma drug efficacy and safety.
- Assess cellular and serological immune responses.

No single model can serve all purposes. Genetically engineered mice are well suited to facilitate elucidation of molecular pathways in melanoma, uncover novel targets of drug candidates, and verify that drugs 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 thoroughly annotated and rigorously validated for their relevance to human melanoma.
- Establish a mechanism or program that would permit integrated preclinical tests using human melanoma models, including relevant cell lines and genetically engineered mice. 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.

VII. Immune Responses

Present status

The occasional spontaneous regression of melanomas has inspired intensive immunological investigations. The studies demonstrated serological response to melanoma antigens, which were expressed either at the cell surface or internally, as well the activation of lymphocytes that inhibit the outgrowth of cultured melanoma cells *in vitro*. Molecularly defined melanoma antigens provide an excellent source of targets for tumor vaccination and for the transfer to melanoma patients of tumor-selective T lymphocytes that were expanded *in vitro*.

Some of the most current immunotherapeutic investigations for melanoma are focused on tumor-reactive T cells, in which melanoma patients received *in vitro*-expanded, autologous T lymphocytes with reactivity against cultured melanoma cells. Localization of tumor-selective CD8⁺ T cells to melanoma cells has been observed. Clinical responses have also been seen, including long term complete remissions. However, the *in vitro*-expanded T cells are often rapidly eliminated *in vivo*, indicating that we need to learn more before making this mode of therapy part of the clinical mainstay. It is possible that better clinical responses will be observed by combining the transferred T lymphocytes with tumor vaccination and/or with an agent that interferes with mechanisms that downregulate anti-tumor responses.

Current efforts are also focused on therapeutic vaccination, which boost immunity to melanoma. In one approach the tumor vaccines are composed of dendritic cells that have been transfected, “pulsed”, or fused to tumor cells to present tumor specific antigens. Alternatively, the vaccines are composed of inactivated pooled allogeneic melanoma cells that present tumor antigens *in vivo* after processing by dendritic cells. A modification of this approach is the use of tumor cells that have been modified to produce lymphokines, particularly GM-CSF, to initiate a more potent immune response. These approaches have produced clinical responses sufficient to merit randomized clinical trials. But the results of one phase III trials in the adjuvant setting which have yet to show consistent benefit.

More recently, new knowledge has been incorporated into the design of vaccine trials, such as our understanding of the polarization of the immune response and its potential regulation through signals that co-stimulate initiation and expansion of anti-tumor immunity. The most

important receptor is likely CD28, but additional receptors such as CD2, CD137, CD83, and CD40 are also involved. Finally, gene-based vaccines are also being evaluated because they are easier to produce and standardize. They have been constructed in the form of recombinant viruses or combinations of cDNAs that encode tumor antigens together with immunostimulatory molecules.

Normal processes that function to prevent immunity to self-antigens appear to enable tumors to escape from immunological control. Downregulation of these immunoregulatory processes through blocking CTLA4 signaling or depletion of immunoregulatory T cells (formerly called suppressor cells) may influence the outcome of immune stimulation with vaccines and cytokines. Efforts to determine how to shift the balance of immune stimulation in favor of tumor recognition and destruction are needed.

Recommendations

- Identify the melanoma antigens that induce the best tumor-destructive immune response.
- Establish improved genetic mouse models for immunological studies, including vaccines and combinations of immunotherapies.
- Establish which co-stimulatory signals, in the presence of a source of tumor antigens, most effectively facilitate the generation and expansion of a tumor-destructive immune response.
- Assess which vaccine types most effectively induce a tumor-destructive immune response (gene- and/or protein-based vaccines, cell-based vaccines such as transfected tumor cells, and antigen-pulsed or transfected dendritic cells).
- Identify the downregulatory mechanisms (such as T regulatory cells, CTLA4, and TGF-beta) that play pivotal roles in inhibiting immunity to melanoma antigens and identify how to best overcome this regulation.
- Investigate the effects of current immunotherapy for melanoma on T cell and antibody response to melanoma antigens and correlate the results with clinical outcome.
- Investigate whether infiltration of primary or metastatic melanomas with T cells, and/or expression of melanoma antigens, correlates with clinical outcome.
- Develop new methods for immune monitoring that accurately reflect the vaccine for immune stimulatory cytokine potency and that relate potency to efficacy.
- Employ mouse models to develop procedures for improving the adoptive transfer of tumor-reactive T cells (eg, by combining the T cells with vaccination).
- Investigate how administration of a chemotherapeutic drug may increase the efficacy of tumor vaccination (eg, by destroying T regulatory cells).
- Develop antibodies to melanoma antigens, including those that may induce apoptosis in melanoma cells and various types of antibody–drug conjugates.

VIII. 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 the basic biology of this disease. This is an exciting topic with numerous reports showing promise of utility, but it is not yet clear how robust the approaches will become or what their actual contributions will be. Investigating this area has the potential for large dividends but also for substantial disappointments.

Recommendations

- Assess the significance of melanoma cells in peripheral blood and/or bone marrow. Tumor cells may serve as a significant indicator of poor survival and their characterization may lead to a better understanding of the molecular events that facilitate spread from the primary tumor.
- Establish serum proteomics to determine if alterations in serum proteins can be used to detect various types of cancer. Substantial work remains to be done toward refining techniques and establishing their efficacy and robustness.
- 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.

B. Clinical Research

I. 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 6 to 10 months, less than 5% of patients survive beyond 5 years. In addition, there is no standard care for patients with metastatic melanoma. The range of options includes observation, , surgical resections of limited metastatic disease, palliative resections for local control, 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. Recent efforts have focused on interferon α 2b (IFN), vaccines, or both. High-dose interferon is approved by the FDA 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 its ability to prevent recurrence and death in only a minority of patients at risk. Treating high-risk melanoma patients with vaccines composed of autologous or allogeneic melanoma, cell lysates, or peptide based vaccines are under clinical investigation. While vaccines for melanoma have low toxicity, their clinical efficacy remains to be established. New approaches to adoptive immunotherapy and antibody-mediated therapies to modulate T-regulatory and other cellular components of the immune system figure among the latest interesting developments.

II. New Strategies for Characterization, Monitoring, and Therapy

It is clear that advances in treatment are necessary to improve the lives of patients with melanoma. Basic science and translational research studies will be required to develop treatment strategies and tailor therapies to patients most likely to benefit. Until recently, the most significant difficulty in developing new therapy for melanoma has been the lack of potential targets and agents. Now, some the molecular pathways relevant to melanoma biology have been described. Constitutive activation of the receptor tyrosine kinase (RTK) Ras/Raf/mitogen-activated protein kinase (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 kinase inhibitor with a wide spectrum of targets (RAF, VEGFR1, PDGFR, Flt3, and c-KIT) that inhibit the MAPK cascade. Sorafenib is already in clinical trials and is displaying encouraging clinical activity when used in combination with chemotherapy. Other critical targets must be identified and novel agents must be developed to specifically inhibit them.

Growing knowledge of the molecular biology of melanoma should be used to identify existing agents that target biologic pathways known to be critical to melanoma tumorigenesis and to identify those from new insights into key signaling pathways. 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 and which 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 non-tumor tissue before, during, and after therapy is critical for measuring the effect of treatment and defining predictive features of outcome. Such information can help select patients for specific therapy based on the likelihood of their clinical response or survival enhancement. The analysis of tumor and host tissues for evidence of efficient response to therapy will require developing methods for the optimal use and evaluation

of small amounts of fresh, frozen, and paraffin-fixed tissues samples (eg, RNA- and DNA-based assays) obtained in the course of therapy.

To take advantage of known and to-be discovered critical pathways in melanoma, to improve the survival of patients with melanoma, and to facilitate clinical/translational research efforts in melanoma, we have identified several important areas of emphasis.

Establish tumor/tissue bank and identify biomarkers

Key biomarkers and surrogate endpoints need to be established for epidemiological studies and prevention or therapy trials. The development of biomarkers and surrogate endpoints will require a series of tissue banks—including nevus, tumor, and serum banks linked to complete clinical data. 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 protects 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. First efforts are being made to develop databases that are accessible to all researchers.

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 can be used also to monitor the effectiveness of therapy for individual patients. Consequently, small, more targeted clinical trials could be performed, which seek bigger differences in outcome. This approach would significantly expedite the clinical trial process.

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 non-surgical techniques for obtaining tumor tissue serially.
- Methods for the optimal use and evaluation of small amounts of fresh, frozen, and paraffin-fixed tissues samples (eg, RNA- and DNA-based assays) obtained in the course of therapy.
- Improved imaging techniques (positron emission tomography and magnetic resonance imaging augmented with specific probes) that will provide functional (anti-angiogenesis, immune-mediated mechanisms) and molecular (apoptosis, inhibition of specific signaling pathways) data sufficient to determine the effect of the particular therapy on its putative target (tumor, vasculature, or other) 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.
- Develop multi-center infrastructures to validate biomarkers for melanoma diagnosis, prognosis, and therapy outcome prediction.

III. 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, led by Dr. Doroshow, which has been established by the NCI to improve the clinical trials system and enhance access to therapeutic agents. Of particular relevance to developing new therapies for melanoma 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 also 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 a substantial number of 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 because this disease is less common than others. In addition to these issues, proprietary concerns also limit the use of novel agents in combination, especially when multiple pharmaceutical companies are involved. The development process could be facilitated enormously 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.

IV. 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 enhance the interaction between clinicians and basic research scientists and to coordinate and prioritize laboratory and clinical studies. This working group would include leadership representatives from the SPOREs' Cooperative Groups, including the ACOSOG (given the critical role of surgeons in melanoma treatment), Cancer Centers, NCI, Cancer Prevention, Dermatology, and Pathology. While these communities conduct important and ongoing efforts in melanoma research, there is currently no forum or entity for coordinating or sharing these efforts. This has significantly impeded the efficiency and progress in melanoma research. We envision the Melanoma Working Group as having a distinct role 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. This group could also play an active role in prioritizing research grants. There are only three Skin Cancer SPOREs and much of melanoma research is goals, advising the NCI on important new areas of investigation in melanoma and emerging technologies, new funding opportunities, etc. What is proposed here is not a new consortium, but rather a working group with representatives from established entities to help coordinate melanoma research efforts. Thus the establishment of the Melanoma Working Group should be cost effective. The Melanoma Working Group should foster international links with the leading co-operative melanoma groups such as the EORTC Melanoma Group 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 it available to researchers.
- Create Tumor Tissue Banks composed of pre and post treatment patient specimen to conduct basic and clinical research. Establish guidelines for optimal methods for procurement, preservation, analysis, and distribution. The tumor banks should include primary melanomas and nevi because they provide unique opportunities to study early stages of this disease.
- 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. Vectors and other tools to knock down genes should be available to all interested researchers.
- Develop in vitro screening models for new drugs that take into account the complex tissue environment of tumor cells.
- Participate in RAIDD and other NCI funding mechanisms to more rapidly bridge the gap between the basic research findings and clinical trials.
- Validate animal models suitable for therapy studies.
- Establish a centralized mechanism for production of patient specific therapies, especially cellular therapies, that will facilitate the more broad investigation and standardization of these approaches.
- Repeat vaccine studies in the presence of inhibitors of immune regulation (CTLA 4, NMA chemotherapy, Ontak).
- Establish the Melanoma Working Group as described earlier in this document.
- Develop an international platform

C. Epidemiology and Prevention

I. 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.
- Exposure to ultraviolet radiation, in particular, high levels of intermittent sun exposure. In this respect the role of tanning in tanning parlors and UVA in melanoma development is still controversial and the recent data from ongoing studies may provide more information on this issue.

Prevention of melanoma

Primary prevention has centered on using sunscreens and avoiding exposure to intense intermittent ultraviolet radiation. However, 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.

Secondary prevention, or screening for early stage melanoma, has not yet been rigorously evaluated, but it 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 and a decline in incidence 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.

Common and dysplastic nevi

High numbers of common nevi and dysplastic nevi are well-established major risk factors for developing melanoma. Sun exposure seems to increase the development of nevi and dysplastic nevi among predisposed individuals.

II. Familial Studies

So far, family studies have identified four high-risk susceptibility genes for melanoma. Inherited mutations in *CDKN2A*, which codes for the CDK inhibitor p16INK4, have been shown to confer susceptibility in around 40% of families with 3 or more cases of melanoma and explain the significantly fewer number of families with only 2 cases of melanoma. Mutations in *CDK4*, which confer resistance to inhibition by p16INK4A are very rare. On the other hand, mutated or deleted p14ARF also increases risk for melanoma. Finally, a susceptibility gene has been mapped to chromosome 1p22, but its identity has not yet been determined. Thus, it is becoming clear that novel methods have to be applied to determine other melanoma susceptibility genes in melanoma-prone families.

III. Molecular Epidemiology Studies

The interactions between low penetrance genes and non-genetic risk factors (eg, UV 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*, the receptor for α MSH. These variants increase the proportion of cutaneous red/yellow pigment pheomelanin, and inheritance is predictive of red hair and freckles. These variants also appear to act as low risk melanoma susceptibility genes seen even in people without

red hair.

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

IV. Genetic Studies in Families

We need to better understand the mutation penetrance in melanoma susceptibility genes and the risk of cancers other than melanoma. 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 risk genes) and multiplex families (for high-risk genes). Similarly, genome-wide screens for related traits, such as propensity to develop nevi, are essential because some of the genes underlying these traits may also be melanoma susceptibility loci. An extension of this work would be to conduct targeted SNP genotyping of a panel of low risk candidate genes. Ideally, agreement should be achieved prospectively to screen candidate genes in concurrent large sample sets to avoid drawing erroneous conclusions, which unfortunately occur repeatedly in the literature on all SNP association studies. Large sample sets collected by consortia and large case-control studies can be pooled for analysis (including test and validation sets), as well as those designed specifically to identify genes governing relapse, are urgently needed.

V. 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. Similarly, there is a need to have large-scale population studies to clarify the utility of sunscreens and other sun blockers (ie, specific clothing) in the prevention of melanoma.

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.

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 major melanoma genes identified to date. Additionally, these collaborative studies will enable more accurate estimates of the risk of other cancers in mutation carriers. For example, apart from pancreatic cancer, what is the risk of other cancers in melanoma-prone families? What should be done about screening for these other cancers? Ultimately, such information may translate into better advice for families with multiple cases of melanoma as well as a better understanding of melanoma carcinogenesis.

The identification of low risk genes and the roles of genetic and environmental features 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 GEM study, large cohorts, such as the cohort consortium, and the development of new large case-control and/or cohort studies. Collaborative efforts can develop a robust understanding of the complex and sometimes controversial 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. Interesting opportunities for methodological research include finding ways to join existing data from multiple studies where data were collected in disparate ways, and examining novel bioinformatics and computational approaches for analysis of complex interactions among many risk factors. Such endeavors would be greatly assisted by collaboration between basic science and epidemiology.

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.
- Devise or 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 UV exposure measures, such as ambient estimated UVA, UVB, UVB-erythemal values, self-reported measures of hours of exposure over a lifetime vs. more qualitative measures of UV exposures, and tanning parlor UV exposures over time.
- Develop a method to prioritize scarce resources for genome-wide analyses vs. 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 melanoma in families as well as population-based case series. Determine whether there are constitutively indolent lesions and constitutively aggressive lesions, and whether the disease progression or prognosis 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. In addition, educating groups that focus on body care, such as hairdressers, spa operators and others on the early detection of melanoma may be helpful.
- 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 simple incidence, as an endpoint. This initiative might require coordination among many geographic regions.
- Learn how to educate all segments of the population about melanoma, especially older men who are at higher risk. Support grass root efforts by advocate groups for prevention studies while maintaining a strong rationale for these activities.

D. Training

I. Overview

There is a need to encourage trained physician-scientists to collaborate with basic and other scientists as well as clinicians in melanoma research. This issue is particularly acute in training programs for dermatology with a focus on melanocyte and melanoma biology. Funding by the National Cancer Institute (potentially in partnership with other sister National Institutes of Health institutes, such as 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.

It is urgent to obtain support and establish systems that will optimize the training of new investigators in the basic science, translational, and clinical areas to improve the outcome of patients with melanoma. Within the past five years, several major cancer centers have been established departments of dermatology and departments of melanoma medical oncology. These centers investigate melanoma and skin cancer as a unique discipline. They provide a formal forum for melanoma researchers and clinicians, as well resources and opportunities for specific training in melanoma.

Our major long-term goal is to attract and train researchers at early stages of their careers. The highly skilled and interactive specialists, who understand the unique biology of melanoma cells and the disease in the patient, can work together to reveal and apply critical new information. An immediate challenge is to begin this training for junior scientists, as well as to obtain support for more senior level scientists expressing an interest in melanoma research, but for whom research funding is currently limited.

II. Long-Term Objectives

Goals

- To recruit and train junior physicians, graduate students, and postdoctoral fellows to become competent basic and translational investigators in the field of melanoma research.
- To establish a mentorship program to educate the basic molecular and cellular principles of melanoma biology. This program is designed to offer scientists and physicians the opportunity to become effective melanoma experts by teaching them specific knowledge of human melanoma biology and its clinical challenges.
- 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 MRF solicitations. Applications for T32 pre- and post-doctoral training grants specifically for training in melanoma are to be encouraged and can be expected to lead directly to enhanced recruitment.

Mentorship program

The training objectives may be pursued through initiating a strong mentorship program at the institutions that have a critical mass of senior researchers in melanoma. Junior scientists, students, and faculty-in-training are instructed in the principles and issues of clinical melanoma and basic science studies of human cancer biology with specific emphasis on melanoma. The detailed areas to be taught include melanoma immunology and bio-immunotherapy; melanoma

molecular biology; biochemistry unique to melanin production and response to UV 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 such as behavioral factors associated with melanoma, etiology of primary melanoma, genetic predisposition to melanoma, mechanisms of melanoma invasion and metastases, immunology of melanoma, and melanoma biological heterogeneity.

Solicit funding for melanoma-specific training and career opportunities

Currently, the NIH and DOD support several cancer site-specific pre and postdoctoral training programs but none exists for melanoma. It is critical to have similar funds for training in melanoma.

III. Short-Term Objectives

We need to embrace non-melanoma researchers who are in current funded and non-funded programs as part of the plan to expand our current research base. We need to create an infrastructure to enhance the ability of young investigators to compete effectively for K08, R21 and R01 grants.

We need to expand current mailing lists beyond the initial membership of ongoing group activities. The melanoma research community is too small. It is important to improve the visibility of our research efforts and conferences. This might be achieved by identifying melanoma representative from the dermatology, dermatopathology, and clinical immunology communities to specifically attend the national meetings of these professionals and present information concerning melanoma research opportunities, as well as soliciting names of interested persons to receive further mailings and invitations. The MRF maintains a meeting database that could be mailed to a broad audience on regular basis.

Our members should be encouraged to advertise broadly and early for all meetings that have melanoma sessions or melanoma focus. The series of seminars and educational material can be provided for those who express such an interest.

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.