Transformative Advances in Melanoma Research

Accelerating Scientific Discovery and Translation to Eliminate Death and Suffering Due to Melanoma

HIGHLIGHTS OF THE MELANOMA RESEARCH ALLIANCE
SECOND ANNUAL SCIENTIFIC RETREAT
FEBRUARY 24-26, 2010

MRA MELANOMA RESEARCH ALLIANCE

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Overview

In the U.S. alone, the incidence of melanoma has tripled over the past three decades and is currently one of the top ten causes of new cancers.

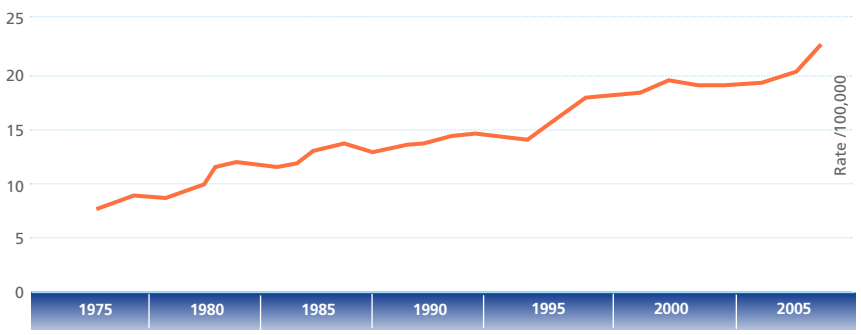
MELANOMA, a cancer of pigment producing cells, is the deadliest form of skin cancer. Its capacity to spread widely to other tissues and organs accounts for those deadly effects. While most melanomas originate from the skin, they can also arise from other parts of the body containing melanocytes, including the eyes, brain or spinal cord, or mucous membranes. Very early stage (localized, Stage I) melanoma is greater than 90% curable with surgery, while patients with disseminated Stage IV melanoma have a median life expectancy of less than one year.

Trends in the incidence of melanoma show that it is one of the fastest growing cancers and is a global public health burden. In the U.S. alone, the incidence of melanoma has tripled over the past three decades and is currently one of the top 10 causes of new cancers. At the same time, despite tremendous advancements in medicine, the death rate due to melanoma has remained static. Approximately one American is diagnosed every eight minutes and one American dies every hour from melanoma.

Only three U.S. Food and Drug Administration (FDA) approved therapies for metastatic melanoma currently exist, and they benefit only a minority of patients. Melanoma poses a difficult challenge for many reasons. Importantly, it is not a single disease that

Figure 1: The incidence of melanoma in the U.S. has almost tripled over the past 30 years. Incidence rate of invasive melanoma per 100,000 in the U.S. 1975 - 2005 (both sexes, all races, age adjusted).

{SOURCE: SEER 9 AREAS (SAN FRANCISCO, CONNECTICUT, DETROIT, HAWAII, IOWA, NEW MEXICO, SEATTLE, UTAH, AND ATLANTA)}



WENDY SELIG
President
Chief Executive Officer, MRA

"MRA is building a robust, collaborative melanoma research community focused on delivering effective results as quickly as possible."

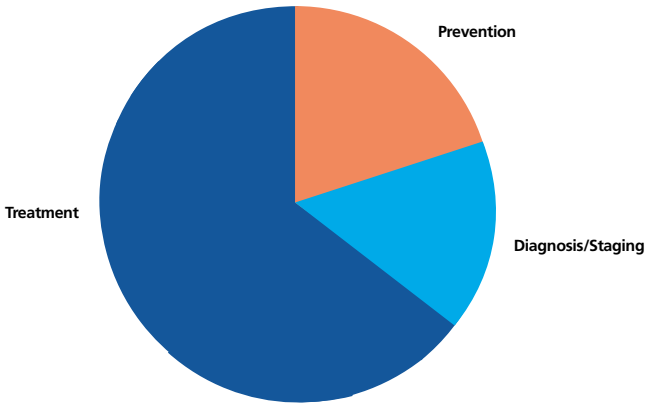
MRA has awarded $17 million to 44 research projects with plans to award an additional $5 million in 2010 to support new and innovative research that will make transforming advances in melanoma prevention, diagnosis, staging, and treatment.

can be traced to a single cause such as a mutation in a particular host gene. Instead, several different genes are implicated in the pathogenesis of melanoma. Moreover, the genetic backgrounds in which those genes are found also can very much influence the outcome of disease and the effectiveness of a particular drug or therapeutic regimen. These complexities put an extra onus on the physicians taking care of patients with melanoma and also the researchers who are developing therapies, diagnostic procedures, and preventive measures.

Despite the challenges that melanoma poses, there are hopeful signs for a better future outlook for patients. New treatments under development, including immunotherapies and molecularly targeted therapies, have produced dramatic responses in some melanoma patients. Even though many clinical responses have so far proved to be transient and thus are not the "ultimate answer" for treating this disease, these results provide proof-of-principle for moving forward. Decades of research in melanoma have translated into significant scientific and clinical advances over the last several years and have generated much excitement in the scientific community and among patients and advocates. With the significant infusion of research funding from the MRA, investigators are finding ways to improve upon these results as well as create new prevention, detection, and treatment approaches to combat this deadly disease.

The Melanoma Research Alliance (MRA) was established in 2007 under the auspices of the Milken Institute, with the generous founding support of Debra and Leon Black. Reflecting the urgency of its mission to accelerate research to end suffering and death due to melanoma, MRA's research portfolio has grown rapidly in its first few years. As of March 2010, MRA has awarded $17 million to 44 research projects with plans to award an additional $5 million in 2010 to support new and innovative research that will make transforming advances in melanoma prevention, diagnosis, staging, and treatment.

FIGURE 2: Of the MRA research funding awarded in 2008 and 2009, approximately 68% was directed to developing new treatments, 17% towards prevention studies, and 15% for diagnosis and staging research.



In less than two years of active research funded by MRA, significant progress has already been made – from studies published in high-impact peer-reviewed journals to patent applications, from presentations at scientific meetings to new cross-sector collaborations. MRA is building a robust, collaborative melanoma research community-focused on delivering effective results as quickly as possible.

Progress and opportunities in melanoma research was the focus of the MRA Second Annual Scientific Retreat, held February 24-26, 2010, in Las Vegas, Nevada. The annual MRA retreat is a key element of MRA's program, facilitating collaboration by bringing together leading scientists from the U.S. and abroad, as well as senior leadership from non-profit foundations, government agencies, industry, and other key stakeholders to share their latest findings and to identify new approaches to understanding and treating melanoma. The retreat featured presentations from MRA-funded investigators, invited special lectures, and focused sessions on key topics of interest. One of these key topic sessions – a panel discussion on combinatorial therapies for cancer will be summarized in a separate paper. This report summarizes the highlights and key themes of the meeting's scientific sessions.

2010 MRA SCIENTIFIC RETREAT HIGHLIGHTS AND KEY THEMES

- Identifying New Therapeutic Targets, Candidate Drugs
- Developing New Immunotherapies and Combined Surgical Approaches
- Genome Scans, Other Strategies for Identifying Melanoma Markers
- New Technologies for Early Melanoma Detection, Prevention
- Nutritional Approaches to Melanoma Research

DEBRA BLACK
Co-Founder
Chair of the Board, MRA

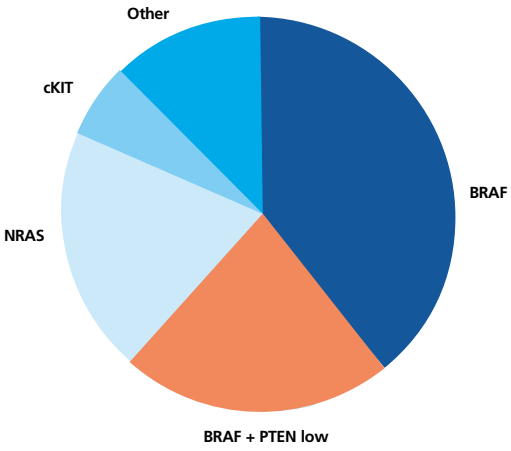
"We measure the value of research based on how it translates into the best options for melanoma patients. The global melanoma research community is poised with innovative and novel ideas that promise to find a cure for melanoma. We are pleased to be able to support these incredible efforts and spur the development of even more effective approaches."

Identifying New Therapeutic Targets, Candidate Drugs

Researchers aim to identify specific molecules critical to tumor initiation and progression with the goal of interfering with these pathways through targeted cancer therapies. Important genetic changes identified in melanoma tumors include those in NRAS, BRAF, PTEN, and cKIT.

FIGURE 3: Distribution of somatic genes associated with melanoma risk

{ COURTESY OF KATHLEEN DOHONEY }



The pathway in which GNAQ and GNA11 operate will prove a clinically important target for drugs with which to treat a substantial group of individuals with uveal melanomas.

A NEW ONCOGENE PROVIDES OPPORTUNITY FOR THERAPEUTIC INTERVENTION IN OCULAR MELANOMA

G protein on the cell surface normally transmit external signals into the cell under controlled conditions, leading to cellular activation; if mutated, however, they can remain locked in the "on" setting, leading to malignancy. A G protein subunit, called GNAQ, is frequently mutated among uveal melanomas that occur in the eye. Such melanomas have a distinct biology, leading to detached retinas and frequently metastasizing to the liver. Although mutant GNAQ accounts for a substantial subset of uveal melanomas, it is by no means responsible for all of them, according to Boris Bastian of the Memorial Sloan-Kettering Cancer Center, and his collaborators.

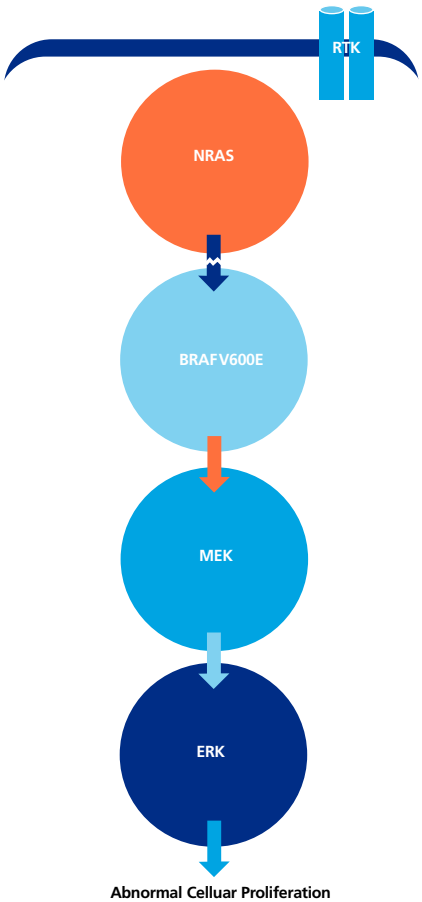
Bastian and his team recently identified mutations in another, functionally related, G protein subunit among melanomas of the eye, which is called GNA11. Importantly, GNA11 presents itself as a separate potential target for treating this subset of uveal melanomas. In very early testing, an inhibitor of MAP/ERK kinase (MEK), which aims specifically at a step late within the cascade of biochemical reactions is partly overseen by this family of G proteins, proved helpful to one patient with GNAQ-mutant melanoma who was tested so far, resulting in disappearance of the patient's metastases. This singular clinical finding, although provisional, is a hopeful opportunity for a mechanism-based approach. Future drug development efforts should target the

pathway closer to the mutant gene. Bastian and his collaborators believe that the pathway in which GNAQ and GNA11 operate will prove a clinically important target for drugs with which to treat a substantial group of individuals with uveal melanomas and plan to look for and evaluate inhibitors of this pathway.

REGRESSION OF MELANOMAS IN PATIENTS BY INHIBITING ACTIVATED BRAF

Approximately 40-60% of cutaneous melanomas have mutations in the BRAF gene, which encodes a protein that plays an important role in the MAP kinase signaling pathway involved in cell proliferation and differentiation. Paul Chapman of Memorial Sloan-Kettering Cancer Center, along with an international team of academic and industrial researchers, is evaluating PLX4032, an experimental drug that binds to mutant BRAF (V600E). Tumors regressed and symptoms improved among patients who received PLX4032 in a Phase I trial. These clinical findings provide a proof of principle, showing that an inhibitor that is directed against a molecular target implicated in melanoma can dramatically interfere and arrest this cancer in at least a subset of patients. "We now have a path," Chapman says.

FIGURE 4: A simplified schematic of the BRAF signaling pathway in melanoma illustrates that in the presence of the BRAF(V600E) mutation, the MEK/ERK signaling cascade is abnormally activated, which causes increased cellular proliferation and tumor growth.



Approximately 40-60% of cutaneous melanomas have mutations in the BRAF gene.

To date, responses have been seen in 80% of patients with BRAF (V600E) mutation, including rapid symptom improvement in some patients. Responses included shrinkage and reduced metabolic activity of liver and lung metastases as well as bowel and bone metastases. Median progression-free survival is at least seven months. Those receiving PLX4032 experienced relatively mild side effects, including rash and fatigue, while some individuals developed benign skin lesions, called keratoacanthomas (KAs), or treatable squamous cell cancers. Despite the positive response rates, it is too soon in terms of clinical evaluation to determine whether this candidate drug prolongs overall survival of melanoma patients. Clinical development of this drug continues to proceed, and an international randomized Phase III clinical trial has begun. Future clinical development should include strategies to rationally combine PLX4032 with other drugs to further increase the clinical response and avoid drug resistance.

Even with these early successes in testing PLX4032 in melanoma patients, Neal Rosen of the Memorial Sloan-Kettering Cancer Center offers a cautionary example of the complexities with which such inhibitors affect molecular targets in the BRAF and similar signaling pathways. His recent research findings help to explain at the molecular level "why PLX4032 works." They also provide "possible reasons for tumor progression on PLX4032, when it occurs," he says. While PLX4032 selectively inhibits downstream MEK/ERK signaling and cellular activation in mutant-BRAF cells, it paradoxically activates MEK/ERK signaling in cells with normal BRAF. It thus has the potential for inducing carcinogenesis in cells lacking the BRAF V600E mutation.

These clinical findings are a proof of principle, showing that an inhibitor that is directed against a molecular target implicated in melanoma can dramatically interfere and arrest this cancer in at least a subset of patients.

Not only do the findings emphasize the need to select patients who have BRAF-mutant cancers for current and future clinical studies of BRAF inhibitors, they also underscore the enormous molecular and genetic complexity underlying the biology of melanoma and the consequent need to evaluate new and promising tumor inhibitors with utmost care.

ETV1, A NEW CANDIDATE ONCOGENE IN MELANOMA

Additional complexities are emerging. Levi Garraway of the Dana-Farber Cancer Center and his collaborators recently identified ETV1 (ETs variant protein 1), a nuclear transcription factor affecting downstream signaling cascades, as a possible novel molecular target for melanoma inhibitors. Changes in this molecular target also occur in other cancers besides melanoma, including prostate cancer, he says. Moreover, findings from several sets of experiments focused on ETV1 in rodents are consistent with its role as an oncogene in melanoma. Garraway and his team are beginning to identify inhibitors of ETV1 or other targets in this same pathway as part of an early stage in developing novel agents for eventually treating melanoma patients.

A PATHWAY TO RATIONAL DEVELOPMENT OF COMBINATORIAL THERAPIES

Chapman, Rosen, Garraway, and others emphasize the importance of going beyond identifying single drugs to find synergistic drug combinations to use in treating

melanoma patients. Tumors treated with targeted therapies sometimes shrink or stop growing, but then recover and resume growth after a period of only weeks or months. The ability of cancer cells to survive treatment allows the emergence of cells that are resistant to treatment. Michael Weber of the University of Virginia has shown that melanoma cells can develop "compensatory changes" during targeted drug treatments and those changes can undermine the initial effectiveness of those drugs. Thus, it makes strategic sense to treat melanoma patients with two or more drugs that are directed against different targets as a way to achieve therapeutic synergy and thus prevent or forestall the development of compensatory changes that can lead to drug resistance.

Weber and his collaborators are examining how combinations of drugs affect different representative melanoma cell lines, looking for evidence of drug interactions that could provide a rational basis for developing combination therapies. Diclofenac and other non-steroidal anti-inflammatory drugs like celecoxib and ibuprofen appear to sensitize melanoma cells to the inhibitory effects of sorafenib, pointing to an unanticipated interaction between cyclo-oxgenase signaling (inhibited by diclofenac) and the MAP kinase pathway (inhibited by sorafenib). Weber suggests that if the mechanism underlying this interaction were better understood, it might lead to a novel and clinically useful approach to treating some melanoma patients. It also points to possible effects on drug response that might be observed in people taking ibuprofen and other anti-inflammatory medications.

Scientists emphasize the importance of going beyond identifying single drugs to finding effective drug combinations to use in treating melanoma patients.

Developing New Immunotherapies and Combined Surgical Approaches

Melanoma is the most immunogenic human cancer and can induce specific cellular and serological anti-tumor immune responses in melanoma patients which are potentially capable of eliminating tumor cells. Thus, investigators are seeking ways to stimulate the host immune system to combat melanoma. This particular cancer is a model for mobilizing immune responses against not only melanoma but also many other malignancies, says Victor Engelhard at the University of Virginia.

NEW ANTIGENS TO ENHANCE IMMUNOLOGICAL CONTROL OF MELANOMA

T cells respond to intrusive, foreign, or otherwise abnormal agents, such as infectious pathogens or cancer cells, by recognizing and responding to signature molecules called antigens. The antigens recognized by T lymphocytes are derived from proteins and converted by degradation processes in cells into smaller components called peptides, which are displayed on the cell surface. In melanoma immunotherapy development, a challenge is that many antigens targeted to date are not required to support cellular transformation, proliferation or metastasis. Thus tumor cells can escape immunotherapy.

Engelhard and his collaborators have described a new cohort of melanoma-associated antigens that consist of peptides that are further modified to contain phosphate groups, providing a "hook" for biochemical isolation and identification. These antigens are derived from phosphorylated cellular proteins, many of which are associated with vital signaling pathways. In an experimental system using a mouse xenograft model, these phosphate-containing peptides can stimulate T-cell immune responses, controlling the growth of melanoma and thereby prolonging the survival of the animals, Engelhard says. Moreover, by tinkering with the chemistry of those peptides, it becomes possible to elicit an even more potent immune response in some of the mice being tested. Phosphopeptides can also stimulate human T cells. These findings are a promising step toward developing a kind of "cancer vaccine" to control melanoma in patients with this disease.

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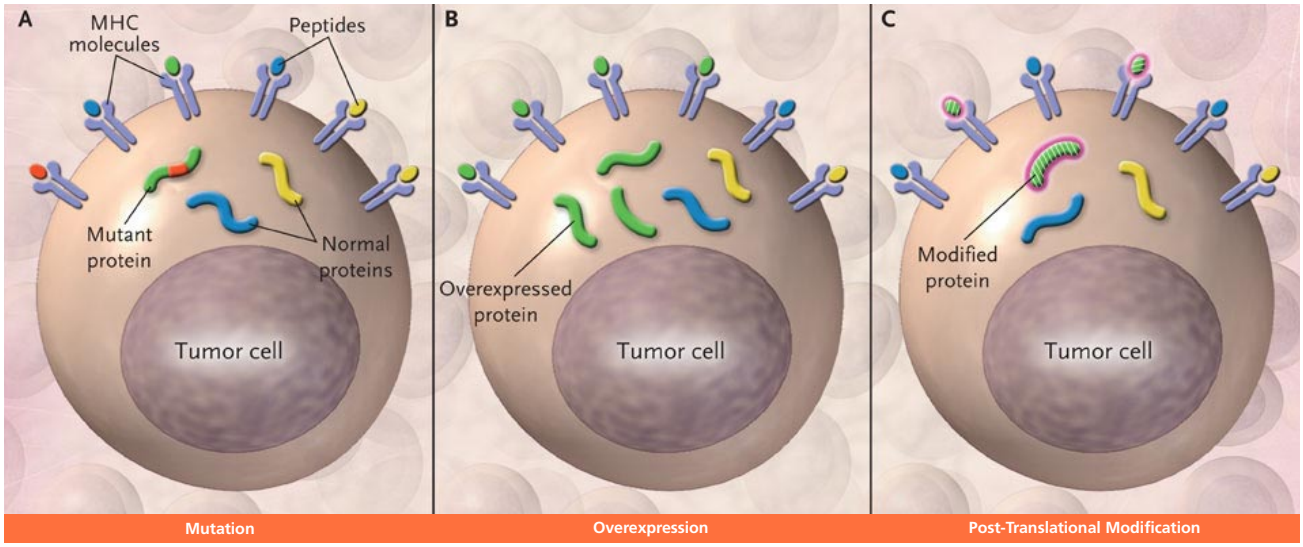
PEPTIDE-BASED PLATFORM FOR ENHANCING T-CELL THERAPY FOR MELANOMA

Ton Schumacher of The Netherlands Cancer Institute and his collaborators are also working on peptide antigens that are associated with melanomas. As part of their effort, they are seeking to identify which of these antigens are recognized by tumor-infiltrating lymphocytes (TIL)—cellular components of the immune system. By examining which peptide antigens are recognized by TIL from individual melanoma patients, and correlating these findings with clinical outcomes, they are hoping to determine which melanoma-specific T cell responses are most valuable. To achieve this they are combing through a collection of more than 200 melanoma-associated, patient-derived peptide antigens, utilizing a new assay system that they have developed. This approach could eventually lead to refined, TIL-based treatment procedures, according to Schumacher.

Figure 5: What does the immune system recognize on tumor cells? Three ways for self antigens to become tumor antigens: mutation, overexpression, and post-translational modification.



PROGRESS IN CTLA-4 BLOCKADE AND NEED FOR NEW BIOMARKERS

In yet another approach to stimulating a fuller host immune response against melanoma, Jedd Wolchok of Memorial Sloan-Kettering Cancer Center and his collaborators are using the monoclonal antibody ipilimumab to augment T-cell responses in patients with metastatic melanoma. Ipilimumab, currently under clinical development in Phase III trials, works by blocking the CTLA-4 inhibitory signal to T cells, thereby "releasing the brakes" on anti-melanoma immunity. About 12-15% of melanoma patients with Stage IV disease who have previously not responded to other treatments experience significant tumor regressions on ipilimumab therapy, and others experience disease stabilization. Some patients survive two years or more after beginning treatment, Wolchok says.

Efforts are under way to better understand the reasons for differences in response among melanoma patients and ways of identifying those patients most likely to benefit from anti-CTLA-4 therapy. Among the notable differences found so far between individual patients who do or do not respond to this monoclonal antibody are those in total circulating lymphocyte numbers after treatment, and the presence of serum antibody responses to the cancer-associated antigen, NY-ESO-1. These efforts to augment immune responses in melanoma patients are complicated because of immunologic differences within individual patients and between one metastatic tumor and another, according to Wolchok. For example, otherwise similar metastatic tumors within an individual patient may have distinctive immunological signatures and microenvironments, making it difficult for the patient's immune system to act equally well against them both at a given point in time. This may explain why some patients have 'mixed responses' to immunotherapy, with some tumors decreasing or disappearing while new tumors appear or others get larger. The most important endpoint for immunotherapy trials should be the 'gold standard' of overall survival.

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ROLE OF ICOS EXPRESSION IN ANTI-CTLA-4 THERAPY

Meanwhile, Padmanee Sharma at the M.D. Anderson Cancer Center and her collaborators are looking at inducible costimulator (ICOS) as a potentially useful marker for identifying and then following the clinical course of the subset of melanoma (and other cancer) patients most likely to respond to anti-CTLA-4 therapy. CTLA-4 blockade is associated with the expression of ICOS on CD4 T cells in the peripheral blood and tumor tissues. Ongoing studies will determine whether ICOS stimulation could broaden the usefulness of CTLA-4 blockade as a means of controlling melanoma.

MODULATION OF LYMPHOCYTE MIGRATION TO ENHANCE IMMUNOTHERAPY

Host immune responses often fail to control disseminated melanoma because the tumor-killing T cells –CD8 T cells – are incapable of infiltrating metastatic lesions, points out David W. Mullins of the University of Virginia. In both patients and mouse models, metastatic lesions may be devoid of infiltrating CD8 T cells, likely because the

T cells are programmed to exclusively seek out and destroy melanoma in the skin, while ignoring tumor in other anatomic sites. However, Mullins and his collaborators demonstrated that T cell infiltration of metastatic lesions can be induced or restored through modulation of the molecular machinery that regulates T cell targeting. In patients, infiltration of metastatic tumors and positive clinical prognosis correlates with T cells that have a specific chemo-attractant receptor – CXCR3 – on their surface. In mouse models, activation of CXCR3 on T cells and induction of the CXCR3-attractant factors in the tumor can overcome deficiencies in T cell infiltration of metastatic tumors, thus maximizing the effectiveness of cancer vaccines and adoptive T cell transfer therapies.

ENHANCING THE CD4+ T-CELL SUBSET TO IMPROVE IMMUNE RESPONSES TO MELANOMA

Another approach to enhancing host immune responses entails stimulating CD4+ "helper" T cells to increase the activity and penetration of CD8+ "killer" T cells into tumor sites, says Timothy Bullock of the University of Virginia. While it is generally accepted that helper CD4+ T cells play a pivotal role in any productive CD8+ T cell response, relatively little attention has been paid to developing vaccination strategies to expand CD4+ T cell responses against tumor antigens. Dr Bullock has found that multiple interventions are necessary to overcome the normal limitations on CD4+ T cell responses against tumor antigens. So far, these approaches, which are being evaluated in mice, appear to have potent effects in terms of driving more CD8+ T cells into melanoma tumor sites and thus gaining better control over their growth. The longer-term goal is to adapt this approach for use in cancer vaccines to treat melanoma patients.

Another challenge in attempting to optimize host immune responses against melanoma comes from dealing with the delicate natural balance between protective immunity and tolerance.

TARGETING MFG-E8 AS MELANOMA THERAPY

Another challenge in attempting to optimize host immune responses against melanoma comes from dealing with the delicate natural balance between protective immunity and tolerance, according to Glenn Dranoff of the Dana-Farber Cancer Institute. For example, the cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF) has dual roles in immunity, acting in both tolerance and protective immunity depending on the host state. Dranoff and colleagues have identified the milk fat globulin MFG-E8 as a factor in the tumor microenvironment which might skew GM-CSF activity towards disease promotion rather than inhibition. Thus MFG-E8 blockade might prove beneficial in cancer immunotherapy. Dranoff and collaborators are also using implantable scaffolds to deliver several types of molecular factors, including GM-CSF, to specific tumor sites in mice. The scaffold can control the delivery of those agents both spatially and temporally, he says. Thus, it becomes possible to optimize the effects of those time-released agents on host dendritic cells, boosting their anti-tumor activity and protective immunity. For example, following such treatment, there was "striking regression" of B16 tumors in mice. He anticipates taking this approach, once it is optimized in mice, into clinical trials.

SURGERY PLUS IMMUNOTHERAPY AS INITIAL THERAPY FOR STAGE IV MELANOMA

In a clinical approach that depends in part on immunotherapy, Donald Morton of the John Wayne Cancer Institute is assessing the combination of surgical resection and immune-system stimulation as a way of combating stage IV metastatic melanoma. He notes that surgery alone in this group of patients seems to show some benefit. Both Canvaxin (a vaccine consisting of irradiated melanoma cells) and BCG (a vaccine against tuberculosis) have been tested for their ability to prolong survival among patients with metastatic melanoma, and it is possible that BCG by itself is immunotherapeutic in this patient population.

Morton is currently overseeing an international, multicenter randomized clinical trial to compare surgery alone to surgery plus BCG vaccine or to "best available medical therapy" as initial treatment for stage IV metastatic melanoma. Patients with six or fewer metastatic sites in no more three organs will be eligible to participate in this trial, which is expected to enroll approximately 400 patients over four-five years.

Genome Scans, Other Strategies for Identifying Melanoma Markers

A major goal of current melanoma research is to determine which genes and genetic markers at the molecular and chromosomal level or other characteristics at the individual level—such as propensity to form moles, fair skin, and eye and hair color—affect one's overall risk for melanoma. That risk is thought to be about 50 percent genetically determined, while the other half is environmentally driven, with exposure to ultraviolet radiation being a major risk factor.

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GENOME-WIDE ASSOCIATION STUDY TO IDENTIFY MELANOMA PREDISPOSITION GENES

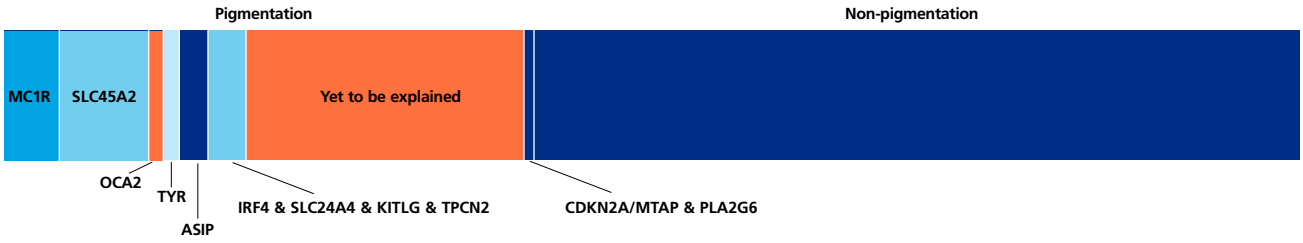
Nicholas G. Martin of the Queensland Institute of Medical Research in Australia and his collaborators there and in England (as part of the melanoma genetics consortium, GenoMEL) are conducting several genome-wide association studies to identify genetic markers that will help in determining which populations and what individuals are most at risk for melanoma. This analysis depends on looking at single nucleotide polymorphisms (SNPs) across the entire genome, and melanoma-associated abnormalities have been found on chromosomes nine, 20, and 22. Additionally, there are 20 or more pigment-related genes, including IRF4 (interferon regulatory factor 4), which appear to influence melanoma risk.

MELANOMA STEM CELLS AS THERAPEUTIC TARGETS

The search for melanoma markers is also taking place at the cellular level, following several distinct strategies. For instance, Jonathan Cebon of the Ludwig Institute for Cancer Research and Melbourne Center for Clinical Sciences and his collaborators are

combing through melanoma cell colonies in vitro, finding that they are heterogeneous, and seeking to identify stem cells within those mixed populations since these might have the most "lethal, proliferative potential." A key question is whether such a subset of cells within a melanoma tumor might carry different sensitivities to drug and immunological treatments as well as express different targets than do the bulk of cells. Although cells carrying the CD133+ marker (a marker used to identify cancer stem cells) account for only about 1 percent of the cells within melanoma cell lines in his studies, these cells tend have a much higher capacity for melanoma colony formation in vitro Cebon says. In one well studied cell line, CD133 expression was tightly linked to expression of the cancer testis antigen NY-ESO-1, suggesting a potential target for melanoma stem cell based therapies. NY-ESO-1 appears to have prognostic significance in melanoma. However, both CD133+ and CD133- cells showed similar tumorigenic potential when tested in mice, in which their seemingly key in vitro differences vanish. This disappearance suggests that environmental signals in the host can override characteristics that appear prominent in vitro. Meanwhile, Cebon and his collaborators also are developing additional strategies for identifying stem-like cells in melanoma and developing a list of genes that are expressed in these cells as another way to identify potential novel targets for treating melanoma.

Figure 6: Relative contribution of pigmentation and other inherited genes to melanoma risk.
{ COURTESY OF NICHOLAS MARTIN }



GENETIC ABERRATIONS IN MELANOMA METASTATIC DIVERSITY

Daniel Pinkel at the University of California, San Francisco, is searching for genes that would mark the relative propensity of melanoma cells to form metastases. He is analyzing clinical specimens, comparing the relative expression patterns of genes in primary tumors versus metastases. Because the specimens were fixed in formalin and then embedded in paraffin, there is considerable "noise" across all the samples rendering analysis difficult, he says. Various algorithms are being employed to correct for this noise and identify true differences. Although he can measure some changes in metastatic profiles of patients over time, such as changes in gene copy numbers, additional data will be necessary to understand how those changes might affect outcomes.

GENOMICS, DRUG SCREENING, AND INFORMATICS TO IMPROVE PROGNOSTIC INDICATORS

In yet another search for markers of metastatic outcomes, Dave Hoon of the John Wayne Cancer Institute and his collaborators are examining 40 defined cell lines

derived from melanoma patients with stage III (regional) metastatic disease. For comparison sake, those lines are categorized as being derived from patients with either relatively good or poor prognosis. Another set of seven cell lines derives from patients with brain metastases. The research strategy depends on the use of expression and genomic microarrays to search for significant aberrations in gene expression that could prove to be biomarkers for clinical outcomes and potential therapeutic targets. When gene expression and patient survival data are combined, the analysis yields several transcription, cell cycle, and DNA replication factors as being associated with survival and thus as potential biomarkers. Genomic aberrations such as deletions and amplifications were also analyzed and compared to gene expression analysis to determine major pathway changes in good versus poor prognosis. Once further refined, these biomarkers could prove useful clinically for guiding treatment decisions for individual melanoma patients.

Hoon and his collaborators also are looking at SNPs that might serve as markers for patient prognosis. At this stage of analysis, they have identified several chromosomal "hotspots," including on chromosomes six and 15, where there are noteworthy loss-of-heterozygosity (LOH) sites. If these or other LOH sites can be validated, they might prove useful as biomarkers, particularly because they appear also in serum samples, making it possible to develop diagnostic blood tests, thus sparing melanoma patients from undergoing more invasive procedures.

Genomics is a powerful tool to provide knowledge for cancer prevention, detection and treatment.

MAPPING THE MELANOMA GENOME

Genomics is a powerful tool to provide knowledge for cancer prevention, detection and treatment, says Lynda Chin of the Dana-Farber Cancer Institute. Although several key genetic mutations have been identified in melanoma, there are likely many more to be discovered that may play important roles in the etiology and progression of the disease.

The Cancer Genome Atlas (TCGA) project, which was established in 2006 under the support of the U.S. National Institutes of Health, recently formed a Melanoma Working Group as part of this very broad-based effort in cancer research. The goal of TCGA is to generate an atlas of genomic alterations for each of the 20 human cancers it will characterize in the current phase, and such atlases will serve as a public resource that enables cancer research in basic, translational to clinical arenas. The project is mandated to release data on a timely basis pre-publication and in a useful form to the research community.

Researchers working on TCGA hope that through integrative analyses of complex genomic data linked to clinical annotations, altered genes that account for tumor establishment, its tendency to form particular metastases, targets for therapy, and markers for tumor behavior and patient outcomes can be identified. However, recognizing that hundreds and perhaps thousands of genes are apt to be changed during tumorigenesis, downstream functional studies beyond TCGA will be required to cull "passengers" from "drivers", Chin Says.

New Technologies for Early Melanoma Detection, Prevention

Although melanoma is typically curable if identified early, there are no official recommendations for screening the general population for melanoma. However, individuals who are at high risk, including those with fair skin or plentiful moles, are advised to take extra care in terms of restricting exposure to sunlight and to examining skin regularly. Yet, despite this ad hoc approach to screening for melanoma, perhaps as many as 3.5 million suspicious skin lesions are removed each year to discover about 116,000 melanoma cases, according to Allan Halpern of Memorial Sloan-Kettering Cancer Center.

Several efforts are under way to improve established technologies and to develop novel devices for detecting early-stage melanomas and distinguishing them from benign lesions, particularly for high-risk individuals. Although used by only about 25-50 percent of U.S. dermatologists, for example, **dermoscopy** provides a straightforward means for magnifying skin lesions about 10-fold and visualizing diagnostic clues not visible with the naked eye during routine examinations, according to Laura Korb Ferris at the University of Pittsburgh.

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One recent improvement in melanoma detection utilizes **multispectral imaging** to create images highly analogous to dermoscopy. The system utilizes multiple wavelengths of light, some of which penetrate deeper within skin tissue, Ferris says. The resulting multiple images of single lesions undergo computerized analysis, which can then predict the likelihood that a lesion is malignant, she says. A commercial version of this system is under regulatory review.

Real-time (RT) confocal microscopy is a major step beyond dermoscopy, providing higher resolution quasi-histologic images, according to Kelly Nelson of Duke University. An advantage over dermatoscopes or histology is that skin lesions can be examined in situ to a depth of about 0.44 mm without doing biopsies or causing any local tissue damage. Indeed, RT confocal imaging can guide clinicians as to where exactly to remove biopsy samples for subsequent analysis, while it also provides a direct sense of how skin tumors appear and behave in individual patients.

Another approach to visualizing skin lesions takes advantage of remote-sensing technology by applying it to produce and analyze whole-body images, according to Clara Curiel at the Arizona Cancer Center. The University of Arizona-Raytheon academic-industrial partnership has developed a proof-of concept on a Skin Change Detection (SCD) system, to map human skin lesion changes using **total body digital photographs (TBDP)**. While TBDP documents skin lesions at one point in time, the proposed SCD system strives to automate the process of quantitatively mapping changes over large areas of skin with time.

One non-visual system for detecting and distinguishing among different types of skin lesions relies on **electrical impedance spectroscopy**, using an externally applied electrode to identify those lesions that warrant further evaluation by biopsy, according to Ulrik Birgersson of SciBase AB and the Karolinska Institute in Sweden. The electrodes probe skin to four different depths, and the differences in impedance that are measured from normal, benign, and malignant lesions are substantial, he says. A pivotal trial in Europe and the United States will begin this year.

Yet another non-invasive approach for detecting melanoma is **molecularly based technology**. According to William Wachsman at the University of California, San Diego, the method uses DermTech's adhesive tape stripping technology to obtain a specimen of skin overlying the lesion from which minute amounts of RNA are extracted and then analyzed for expression of a group of genes indicative of melanoma. Because such a test characterizes genes actively expressed in melanoma, it might also be used for both diagnosis and for identifying drug targets, he says.

Nevertheless, because of the relatively low prevalence of melanoma in the populations likely to be tested and the unproven benefits of these tests for the populations on which they will be used, they may have a limited direct effect on promoting early detection. On the other hand, such technologies may induce physicians, other medical caregivers, and at-risk individuals simply to pay closer attention to suspicious skin changes. If that happens, many deaths from melanoma may be prevented, says Martin Weinstock of Brown University, Providence VA Medical Center and Rhode Island Hospital.

Nutritional Approaches to Melanoma Research

While some investigators are working to detect melanoma in its earliest stages, others are intent on preventing its development. One standard approach is to have individuals limit exposure to ultraviolet radiation from sunlight and tanning beds. However, another strategy involves recognizing the potential importance of nutrition for helping to prevent cancer, or to slow or block the growth and metastasis of malignant cells, according to David Heber of the University of California, Los Angeles.

The links between diet and melanoma are not fully understood, but there are several plausible ways in which these factors can influence the development and progression of cancers, either by enhancing their growth or inhibiting it, that may guide melanoma research in this area. For instance, diets that are rich in fats can lead to chronic inflammatory responses, including activation of interleukin-1B, which in turn may activate nuclear transcription factor-kappa B (NF-kB). Together, these events may stimulate progression of melanoma, Heber says.

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The goal of research is to better understand the effects of specific dietary components on the development or prevention of cancer. Meanwhile, a better mix in the diet of omega fatty acids, phytonutrients, flavonoids, antioxidants such as those in green tea, and other mainly vegetarian foodstuffs can help to lower one's risk for cancer and also to curb development, according to Heber. In general, he says, the American diet and obesity tends to put the innate immune system into a "pro-inflammatory state," which can interfere with host-immune responses that might otherwise help to combat melanoma.

Moving Forward

Although melanoma poses many difficult challenges, researchers working at the basic and clinical levels are making significant progress toward developing better diagnostic and preventive measures, improved treatments, and a deeper understanding of this complex and deadly disease. Recent advances in the development of new therapeutic approaches have generated excitement and optimism for a better outlook for melanoma patients.

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One note of consensus is that combinations of drugs and approaches will be required to most effectively treat metastatic melanoma. This view arises from frustration amid repeated half successes and many outright failures with experimental drugs that are evaluated as single agents in desperately ill patients. It is also valuable to consider not only combinations of targeted therapies, but adding immune stimulatory and surgical treatments, particularly when melanoma reaches an advanced or metastatic stage, and determining which interventions are best suited for a particular patient.

Melanoma experts searching in the laboratory for promising inhibitors with which to block aberrant or dysregulated metabolic pathways in melanoma cells, anticipate that some of these inhibitors will become candidate drugs for clinical development. And part of this goal is to identify which inhibitors are promising candidates for combination drug therapy regimens. There also is a concerted search for melanoma markers for use in diagnosing disease, for assessing the relative aggressiveness of a melanoma and its likelihood of metastasizing, and for improving the selection and monitoring of individual patients during treatment. Furthermore, investigators are studying ways to enhance immune-system responses to melanoma, making them more effective at recognizing heterogeneous melanomas and at eliminating sites of melanoma metastases.

The second annual MRA scientific retreat facilitated the information-sharing across research sectors needed to continue to build a robust, collaborative melanoma research community focused on delivering effective results as quickly as possible.

Scientific principles emerging from this work should be relevant and applicable to other kinds of cancers as well. Focused on finding and funding the most promising melanoma research worldwide, MRA supports novel research programs that will advance scientific understanding of melanoma needed to enable the development of effective treatments and accelerate progress towards a cure. It is only with the collective efforts of academic scientists, clinicians, industry, government and patients that we will end suffering and death due to melanoma.

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FOR MORE INFORMATION ABOUT THE MRA AND HOW TO GET INVOLVED, visit the MRA website at www.MelanomaResearchAlliance.org. The website includes additional information about the MRA scientific retreats and the research award program.

Melanoma Research Alliance 2nd Annual Scientific Retreat Participants

FEBRUARY 24-26, 2010

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1101 New York Avenue, NW, Suite 620
Washington, DC 20005
202.336.8935
www.MelanomaResearchAlliance.org

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