Pathways, Progress & Partnerships

Highlights of the Melanoma Research Alliance
Third Annual Scientific Retreat
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Recent scientific discoveries in the complementary fields of molecular biology and immunology have converged to provide the methods and rationale for significant translational advances that will improve outcomes for melanoma patients and those at risk. Discussions of cutting-edge melanoma research results and key policy issues were held at the Melanoma Research Alliance’s (MRA) Third Annual Scientific Retreat, Feb. 16-18, 2011, in Washington, DC.

The annual MRA retreat is an important forum for exchanging ideas, bringing together more than 150 thought leaders from academia, industry, government, business, and philanthropy to share latest findings and forge new partnerships to further advance the field. MRA-funded investigators, including early career scientists, established investigators, and interdisciplinary teams, report on the progress of their work. This report summarizes the highlights of the 2011 meeting, illustrating the progress that has been made as a result of collaborative partnerships to forge pathways towards success in the fight against melanoma.
Melanoma, a cancer of pigment-producing melanocytes, most often arises in the skin but also less frequently in the eye, mucous membranes, brain, and spinal cord. Melanoma is the deadliest of all skin cancers because of its ability to spread widely to other organs and tissues in the body. Trends in the number of new cases of melanoma show that it is one of the fastest growing cancers worldwide. In the United States alone, the incidence of melanoma has tripled over the past three decades, and melanoma is currently among the top 10 causes of new cancers. When caught early, melanoma is successfully treated by surgery. In contrast, those diagnosed with widespread metastatic disease (Stage IV) have a median survival of less than one year. Despite tremendous advances in medicine, the death rate from melanoma has remained static, in part due to the lack of effective therapies for patients with metastatic disease.

The biological complexity of melanoma, coupled with historic underfunding of research on this disease, poses significant challenges for scientists and clinicians developing new tools and treatments for patients. In 2007, the founding of the Melanoma Research Alliance (MRA) created a catalyst that has accelerated the pace of scientific discovery and its translation. As the largest private sponsor of melanoma research, MRA has awarded more than $30 million in funding to date to 73 research programs led by 114 Principal Investigators in nine countries who are pursuing innovative, translational studies to benefit patients and those at risk of melanoma (Figure 1). This investment has yielded significant advances in the biological understanding of melanoma and the development of new and better preventative, diagnostic, and treatment approaches.

With the infusion of funding by MRA, the field has accelerated, creating excitement and hope for a better outlook for patients and
those at risk. In fact, during the writing of this report, the U.S. Food and Drug Administration (FDA) announced the approval of the first new melanoma treatment in 13 years. This treatment, the monoclonal antibody ipilimumab, is the first ever to show a survival advantage in patients with Stage IV disease (Figure 2). In addition, a promising drug targeting the mutant BRAF protein is in late stage clinical development and as of this writing the company has filed a New Drug Application with the FDA.

“As a melanoma survivor, I want to thank all of the researchers for their efforts. This is such an exciting time in the field of melanoma research. While we are thrilled about how much attention melanoma is getting these days, we recognize that we still have a lot of work to do.”

DEBRA BLACK, CO-FOUNDER AND CHAIR OF THE BOARD
Genetically, melanoma is a highly heterogeneous disease. Mutations in key genes confer susceptibility to melanoma and drive its progression. Environmental and epigenetic factors add complexity to the molecular landscape. Characterization of how these alterations contribute to melanoma will facilitate the development of better preventative, prognostic, and therapeutic strategies.
Researchers are starting to address the low frequency, low to medium penetrance variants for the first time.

SUSCEPTIBILITY GENES

An understanding of the genetic factors influencing risk for melanoma is crucial to identifying at-risk individuals and to increasing the effectiveness of early-detection, as early as, chemopreventative, and behavioral interventions. Phenotypic risk factors, such as skin pigmentation, have been extensively characterized. But the few inherited genes known to heighten cutaneous melanoma risk in families (CDKN2A, CDK4, MC1R) do not explain most of the risk for this malignancy. Recent studies suggest that melanoma risk in the general population is probably determined by multiple factors, including rare high-penetrance mutations or multiple medium to low penetrance genes acting in combination (Figure 3).

Genomic screening of families prone to developing ocular melanoma, being conducted by a research team including Kevin Brown at the U.S. National Cancer Institute, recently discovered evidence for new melanoma risk genes on chromosomes 3 and 14. Honing in on mutations at those loci is an area of active research. In addition, researchers have found a novel genetic variant of MITF (E318K) that appears to be a medium to low penetrance rare variant that increases melanoma risk in some cutaneous melanoma families as well as the general population. MITF is a transcription factor and a master regulator of melanocyte cell cycle progression, survival, and differentiation. Functional assays showed that the E318K mutation increases transcriptional activity of MITF. “We’re starting to address the low frequency, low to medium penetrance variants for the first time,” notes Dr. Brown.
ACTIVATION AND PROGRESSION

Depending on expression levels, MITF can either prevent or foster melanoma due to the integral role the wild-type (normal) gene plays in melanocyte development and response to ultraviolet radiation.

David Fisher of Massachusetts General Hospital pointed out that melanocytes seem to be genetically “wired to survive” the environmental exposure to ultraviolet (UV) radiation through various mechanisms, including expression of anti-apoptotic (pro-survival) factors. For example, the UV response of melanocytes includes an upregulation of the lineage specific survival factor BCL2A1, which is regulated by MITF. BCL2A1 is also an amplified oncogene in certain melanomas, and drugs are currently being developed against its family of proteins. BCL2A1 could be a useful drug target for melanoma that might also improve the effectiveness of other melanoma therapies, such as those targeting mutant BRAF, because in vitro assays demonstrate that BCL2A1 oncogenically cooperates with BRAF(V600E).

Ruth Halaban, Michael Krauthammer, and their research team at Yale University have found candidate genetic aberrations through exome and RNA sequencing of samples from patients with metastatic cutaneous, ocular, mucosal, and acral melanoma. While thousands of somatic coding mutations have been detected, only a handful seems to be shared by two or more samples. Emerging targets of interest are GEF and GAP proteins, which regulate RAS, RAF, PTEN, and PI3K, known to be associated with melanoma, as well as genes encoding proteins that govern cell-to-cell contact, migration, and other factors likely to be involved in metastasis (Figure 4).
Mutations in the BRAF gene, including the V600E variant, which is found in 50-60 percent of cutaneous melanomas, are known to drive melanoma. In addition, it has been recently found by Nallasivam Palanisamy at the University of Michigan that gene fusions of RAF1 and BRAF also drive melanoma in a subset of patients. Although rare in melanoma (as opposed to some other cancers), these fusions were recurrent and expressed in melanoma samples that did not have the BRAFV600E mutation. Functional studies demonstrated that over expression of these gene fusions show onco-genic properties, which was inhibited by sorafenib and an MEK inhibitor (U0126). Dr. Palanisamy suspects that the fusion genes lack the regulatory “braking” domains of BRAF, and thus are over active. Screening for such RAF rearrangements could identify cancer patients who may benefit from RAF kinase inhibitor treatment.

**GROWTH FACTORS**

Alexander Levitzki of the Hebrew University of Jerusalem has focused his efforts on the IGF1R/IRS1-2 signaling pathway as a target for cancer therapy. His research group is testing compounds, called novel tyrphostins, which inhibit IGF1R kinase activity and target IRS1 and IRS2 for destruction. They cause significant inhibition of tumor growth in xenograft mouse model systems of both melanoma and ovarian cancer, resulting in increased survival of treated animals.

“These findings establish a new paradigm of signal transduction therapy – the irreversible elimination of a signal transducer that is essential for cancer cell survival,” notes Dr. Levitzki. Clinical trials currently are in the planning stages by a start-up company founded by Dr. Levitzki.

**TUMOR SUPPRESSION**

Natural tumor suppression triggered by genes such as wild-type p53 is often disrupted in various cancers, so some researchers have focused their efforts on understanding the molecular biology of tumor suppression in melanoma. Although close to 90 percent of melanoma patients retain wild-type p53, its tumor suppressor function is compromised by the MDM2 oncoprotein. Blocking the binding of MDM2 to p53 can reactivate the function of p53. Sanjeev Kumar Shangary of the University of Michigan and his collaborators have designed a class of highly potent and orally active small-molecule inhibitors of the MDM2-p53 interaction and show that their lead compounds are highly effective against human melanoma in in vitro and in vivo laboratory models. These agents are in advanced preclinical development and are expected to enter clinical trials in 2012.
METASTASIS BIOMARKERS

Approximately 80 percent of melanoma patients are first diagnosed with localized Stage I or II disease. In these patients, risk stratification is primarily based on Breslow thickness, where patients with thin melanomas have excellent prognosis following complete surgical excision. However, it is recognized that, even among properly excised thin melanomas, approximately 10 percent of the patients will succumb to recurrent or metastatic disease. Molecular biomarkers for metastatic risk are therefore needed to complement standard clinical and pathological parameters in order to better stratify risk and to identify the high-risk subgroup that needs to be followed and treated more aggressively (Figure 5).

Using genetically engineered mouse models with distinct metastatic potential as “extreme cases,” intersecting with genomic data from human primary and metastatic melanoma samples, Lynda Chin’s laboratory at the Dana-Farber Cancer Institute has identified hundreds of genes that are up-regulated or down-regulated in primary melanomas with metastatic potential. Functional genetic screening for invasive activities and rigorous human relevance validation identified six metastasis oncogenes. Of these, HOXA1, which appears to modulate TGF beta signaling, is a promising lead. This finding suggests that a TGF-b inhibitor (already in clinical development) might inhibit metastasis in those patients whose melanomas have this molecular signature. Additional studies will continue to follow up on candidate genes to develop a multi-gene prognostic assay.

Molecular biomarkers for metastatic risk are needed to complement standard clinical and pathological parameters.
Researchers are working to develop targeted topical prevention agents for individuals with mutations in MC1R.

**PET IMAGING FOR MELANOMA STAGING**

Precise staging of melanoma enables more accurate prognosis and treatment decisions. Positron emission tomography (PET) is often used for molecular imaging of tumors. Currently [18F]FDG is the most widely used PET probe for melanoma in the clinic, but there is a need for additional probes that can be more specific and detect smaller metastases. Melanin pigment is typically overexpressed in melanoma cells and thus is an attractive target for a PET probe. **Zhen Cheng from Stanford University** has created imaging probes that were shown to be selectively taken up by melanin-producing melanoma primary and metastatic tumors, including those in the lungs and brain, in mouse models. Clinical testing of one of these PET probes is expected to begin in the near future.

**PATHWAYS TO PREVENTION**

Individuals with mutations in MC1R (associated with red hair) have a 2-4 fold increase in risk for melanoma, because they have an ineffective protective response to UV radiation. Researchers are working to develop targeted topical prevention agents for this high-risk population. **Sancy Leachman at the University of Utah** is finding that sulforaphane, a natural product derived from broccoli sprouts, might improve the skin’s antioxidant response and increase pigment production. Wild type MC1R protects against UV-induced cell death and DNA damage through production of antioxidants in pathways regulated by the transcription factor Nrf2. The idea is that sulforaphane will activate Nrf2, thus restoring the protective pathways (Figure 6).

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**Figure 6: Pathways in the skin response to UV radiation**

Courtesy of Sancy Leachman
When skin biopsies were treated with sulforaphane and then exposed to UV radiation, the compound prevented many of the damaging effects of UV light and diminished the amount of sunburn. Experiments in cell culture also demonstrated increased pigment production. Next steps include testing the compound in a clinical trial, developing biomarkers of response, and evaluating melanoma prevention potential in a mouse model.

MITF can play a role in melanoma prevention by fostering the pigmentation response to UV radiation. Previous research from the laboratory of David Fisher, Massachusetts General Hospital, showed that forskolin and phosphodiesterase inhibitors enabled potent skin darkening and protection against sunburning and DNA damage in genetically engineered “redhead” (MC1R variant) mice. While the specific tool compounds tested so far did not penetrate human skin, they demonstrate a promising strategy, and small molecule screens are being conducted to identify promising phosphodiesterase inhibitors for potential human use.

One of the most effective ways to lower melanoma risk is to reduce exposure to UV radiation. UV appears to trigger the production of beta endorphins in mice, studies in Dr. Fisher’s laboratory has found, and might possibly explain why some individuals are “addicted” to tanning and do not take extra care to avoid this potent carcinogen. Such UV-triggered release of beta endorphins may have played an evolutionarily conserved role in fostering sun-seeking behavior to prevent vitamin D deficiency.

**RESEARCH CONSIDERATIONS**

Cutaneous melanomas can exhibit many point mutations randomly spread over the genome due to damage from UV radiation; thus, large sample sizes and sophisticated comprehensive analyses are needed to ensure the accuracy of genomic findings. Functional studies to determine driver mutations are critical to nominate therapeutic targets. Some genes and their encoded proteins that have been linked to carcinogenesis may play different roles as the cancer progresses, and that activity will be dictated by the cellular as well as genetic context. In addition, environmental and epigenetic factors, such as methylation, contribute to melanoma risk and progression. Because melanomas are so much more deadly once they metastasize than when treated in the earliest stage, additional research is needed to identify and characterize metastasis biomarkers and develop molecularly-based prognostic assays.

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Mutations in key genes can confer greater susceptibility to melanoma as well as play a role in progression. There is tremendous biological variability in melanoma, especially for tumors that first develop in the skin due to damage from UV radiation. However, despite this variability, most of the factors discovered seem to operate on the same core group of cellular pathways. This suggests that treatments targeting those core mechanisms might prevent melanoma or its progression, and several novel treatments have been developed based on new genetic findings. Some targeted treatments are already being tested clinically in patients with melanoma or other cancers, and others are expected to enter clinical testing soon based on encouraging preclinical results in animal models.

WHAT THIS RESEARCH MEANS FOR PATIENTS AND THOSE AT RISK

The U.S. National Institutes of Health (NIH), and the National Cancer Institute (NCI) in particular, is the largest public funder of melanoma research in the United States and is an important partner in the fight against this deadly disease. In addition to extramural investigator-initiated projects and the intramural NIH research program totaling approximately $110 million in annual melanoma research expenditures, Douglas Lowy, NCI’s deputy director, described NIH’s support of several large-scale programs, such as The Cancer Genome Atlas (known as TCGA), of which melanoma is one of 20 tumor types to be sequenced. The NCI Provocative Questions project is intended to assemble a list of important but non-obvious questions that will stimulate the NCI research community and will result in program announcements for new research initiatives. One goal of the MRA is to facilitate research that will be successful in creating a platform that will attract additional funding from more traditional sources, like the NIH. Of the initial $21.7 million awarded by MRA in 2008 and 2009, an additional $12.3 million was secured by the investigators from other sources, the bulk of it from NIH (Figure 7). Investments in melanoma research by the NCI are critical; in fact, melanoma advances are highlighted in the NCI budget proposal for fiscal year 2012.
Melanoma is one of the most immunogenic human cancers, that is, it triggers an immune response that can recognize and potentially eradicate tumor cells. Although melanoma patients have been found to express T and B cell responses specific for melanoma-associated antigens, this reactivity rarely abolishes the cancer on its own. Recent research has provided a better understanding of how tumors may suppress the immune system, and what components of the immune system need to be modulated to generate an effective anti-tumor response. This research has resulted in various immune-based therapies for melanoma that are currently being tested in the laboratory and clinic, including vaccines, immune-modulating monoclonal antibodies, and adoptive cell transfer therapy.
Recent evidence suggests that cancers usurp normal immune regulatory systems (called immune checkpoints), which interfere with anti-tumor immunity. For example, when T cells are activated by coming into contact with tumor antigens, they produce on their surface the receptor CTLA-4, which then provides a negative signal to effector T cells while fostering the production of regulatory (suppressor) T cells (Figure 8). An antibody against CTLA-4 (ipilimumab) has just been approved by the U.S. Food and Drug Administration to treat patients with advanced metastatic melanoma. Scientists are now working to find ways to improve its efficacy by understanding biological differences between responders and non-responders and designing combination therapies (see Combination Therapy section).

Another checkpoint involves the cell surface ligand, B7-H1/PD-L1, expressed on many human cancers, and its receptor PD-1, an inhibitory receptor in the same family as CTLA-4 that is expressed on activated T cells and down-regulates immune responses. Drew Pardoll of Johns Hopkins University and a team of researchers, including Lieping Chen and Suzanne Topalian, found that tumors expressing B7-H1 are protected against immune destruction. After initial demonstration of low toxicity and clinical activity in a Phase I trial of an antibody blocking PD-1 (MDX-1106), an expanded Phase 1/2 trial has demonstrated objective tumor regressions in one-third of patients with advanced metastatic melanoma (as well as lung and...
renal cancer); additional patients had stable disease or mixed responses. Most of the responses have been surprisingly durable even off therapy, lasting as long as two years after treatment ended. These results suggest a long-lasting modulation of immune system function. Preliminary evidence suggests that expression of B7-H1 on the surface of tumor cells may predict response to anti-PD-1 therapy, thereby serving as a biomarker. Ongoing clinical investigations with antibodies blocking PD-1 or B7-H1 will explore the spectrum of activity of these drugs and effective ways to combine them with other treatments for greater efficacy. “The lessons we are learning in melanoma will teach us a lot and break frontiers for other cancers as well,” Dr. Pardoll said.

Studies are revealing interactions between altered pro-carcinogenic signaling pathways and the immune system, which can suppress anti-tumor immune responses. Although dendritic cells are known to induce anti-tumor T cell responses that lead to tumor destruction, within the tumor environment such a response is often lacking, apparently due to overactivation of the MAPK pathway in neighboring tumor cells, says Patrick Ott of New York University. Studies of dendritic cells co-cultured with BRAF(V600E) melanoma cells suggest that MAPK activity suppresses the actions of dendritic cells via alterations in cytokine production, which compromises T cell priming capacity. That suppression can be reversed by treatment with an MEK inhibitor, which blocks MAPK activation. Future work will assess the effects of BRAF inhibitors on immune function.
Inducing complete regressions is key to successful treatment with ACT.

**STIMULATORS OF ANTI-TUMOR IMMUNE RESPONSES**

Research continues to uncover other components of the immune system that might aid destruction of tumors. Several growth factors and agonists have been shown to stimulate the growth, differentiation, and activation of anti-tumor dendritic cells, including GM-CSF and TLR agonists. **Drew Pardoll of Johns Hopkins University** described studies in which a vaccine made by combining TLR agonists with tumor cells genetically engineered to secrete GM-CSF (so-called “TEGVAX”) induced 10-fold increases in the number of T cells infiltrating melanoma tumors in a mouse model, without also increasing the number of T regulatory cells. Clinical tests of the vaccine are expected shortly.

**ADOPTIVE T-CELL TRANSFER**

Adoptive T cell transfer therapy (ACT) is an immunotherapeutic approach in which a patient’s T cells are expanded ex vivo and re-infused into the patient. One approach involves the use of tumor infiltrating lymphocytes (TILs) in combination with a lymphodepleting chemotherapy regimen (Figure 9). Clinical experience with more than 100 patients with metastatic melanoma treated at three different clinical sites has produced consistent response rates of approximately 50 percent or higher (RECIST criteria). Long-term follow-up of advanced melanoma patients treated by **Steven Rosenberg at the U.S. National Cancer Institute** showed that 30 percent of patients receiving ACT with a lymphocyte depleting regimen have survived four to five years after treatment. Most complete
responders received only one treatment, while partial responses tended to be transient. “The name of the game if you want to cure patients is to induce complete regressions,” Dr. Rosenberg said. Research has focused on determining the immune correlates of ACT success in order to improve upon this approach. The type of T cell infused seems to correlate highly with response. For example, it appears that cooperation between less differentiated and more differentiated T cells is needed to mediate both short-term and long-term tumor control. Xue-Zhong Yu, Moffitt Cancer Center, showed that in mouse models, Tc17 cells are less potent than Tc1 cells, but both are effective at eliciting long-term immunity using different mechanisms. Laszlo Radvanyi, M.D. Anderson Cancer Center, presented results showing that the expression and activation of key T-cell costimulatory molecules by TILs is critical in regulating TIL survival and anti-tumor activity. The success rate of ACT could be increased by activating specific costimulatory molecules using agonistic monoclonal antibodies that can be infused after adoptive transfer of TILs into patients, or during the generation of the TIL infusion product in the laboratory. Results presented suggest that the TNF receptor family member, 4-1BB, might be a key costimulatory molecule that can be targeted to improve the success of ACT. Another interesting observation was that higher expression of the costimulatory molecule BTLA by CD8+ T cells was highly correlated with clinical anti-tumor activity of adoptively transferred TILs in an ongoing Phase II clinical trial.

The use of genetically engineered lymphocytes may improve treatment applicability and effectiveness, especially in patients for whom there is difficulty in obtaining adequate tumor specimens to generate TILs.
responses, but most patients had progressive disease within three to six months. **Cassian Yee at the Fred Hutchinson Cancer Research Center** and his team are working to improve an alternative approach using strategies to more effectively isolate tumor antigen-specific T cells from the peripheral blood of patients. The use of immunomodulatory cytokines can lead to the generation of ‘helper-independent’ cytotoxic T lymphocytes (CTLs) with a central memory phenotype; in addition, unique ‘conditional’ superagonistic altered peptide ligands can also be used to produce a more robust tumor-associated antigen-specific T cell response in vitro and potentially in vivo. Preliminary studies using less toxic conditioning (lymphodepleting) regimens combined with antigen-specific CTL from peripheral blood have led to sustained T cell persistence and long-term clinical responses.

“Engineered T cells are drugs with many functions,” points out **James Heath at California Institute of Technology**. In order to assess temporal changes in T cell populations and their functions, thereby better understanding how to improve ACT, a team including Dr. Heath and **Antoni Ribas at the University of California Los Angeles** developed a nanotechnology-based, bar code assay to monitor the expression of key proteins in single T cells in patients undergoing therapy. Currently, flow cytometry is routinely used to interrogate transferred cells, but it only evaluates population statistics, not functional performance. This new technology revealed a functional evolution of T cells during therapy such that the killing capacity of engineered T cells dissipated due to loss of expression of key molecules, while new tumor-specific T cells with killing capacity appeared during the course of the therapy. Their laboratories, in collaboration with **David Baltimore at California Institute of Technology**, are also using cutting-edge technology to discover new tumor-specific T cell receptors. With this clearer understanding of ACT systems biology, scientists can work to better tailor this therapy to improve patient outcomes.
RESEARCH CONSIDERATIONS

Improving the effectiveness of immunotherapies will depend not only on a better understanding of which components of the immune system need to be stimulated or suppressed to mediate a strong anti-tumor response, but on measuring functional changes over time. Developing ways to measure components of this complex system during therapy is important to improve therapeutic approaches.

Genetically engineered anti-tumor T cells may broaden the applicability of ACT. Despite the success of ACT, it is currently only available at three sites as an experimental treatment, and it poses challenges for adoption by commercial providers. However, researchers are pursuing plans to enable licensing of adoptive therapy within a few years.

WHAT THIS RESEARCH MEANS FOR PATIENTS

The immune system is highly regulated and has elements that stimulate responses to foreign elements and damaged cells, as well as elements that inhibit responses in order to avoid damage to normal tissues. Researchers have discovered that tumor cells may avoid immune destruction by co-opting these regulatory components. Investigators have made tremendous progress devising treatments to modulate these regulatory checkpoints to tip the balance in favor of tumor killing. In addition, adoptive T-cell therapy, in which a patient’s own immune cells are grown and infused back into patients, can be an incredibly effective treatment for some, although it is currently only available at a few cancer centers as an experimental treatment. Not surprisingly, side effects of these approaches are immune-related and some can be serious. Ongoing research is investigating how to improve upon these approaches to increase response rates through identifying biomarkers associated with favorable treatment outcomes.
Melanoma is an ideal case study to address the challenges and produce creative solutions for roadblocks to new drug approvals.

CREATIVE SOLUTIONS

Roadblocks to new drug approval can arise due to differences in criteria between U.S. regulators compared with Europe and other geographic areas in which the drug will be marketed. The design of clinical trials is an area of intense discussion in the community, and many new factors are emerging in an era of personalized medicine. In melanoma, it is likely that trial design will change as new melanoma drugs are approved to serve as comparators. It is clear that enhanced communication between all parties is desired to increase transparency and avoid duplication of failed efforts. The best way to accomplish this, however, is unclear. These issues are relevant to many other cancers; thus, melanoma is an ideal case study to address the challenges and produce creative solutions.
Progress has been made in developing single agent treatments (monotherapies) that target specific molecular aberrations fostering melanoma growth or that target components of the immune system to aid tumor destruction. But both laboratory and clinical studies are finding that resistance often develops, particularly to molecularly targeted therapies, leading to the outgrowth of recurrent tumors. A better understanding of biological pathways has led researchers to design rational combinatorial strategies using immunotherapies, molecularly targeted agents, standard chemotherapies, and anti-angiogenesis drugs.

**Developing Combination Approaches for More Effective Treatment Strategies**
Cancer treatment often must be directed at a “moving target” because of the genetic instability of tumors that enables them to continually evolve strategies for survival. Understanding those pathways, including both de novo and acquired resistance, can inform treatment strategies aimed at overcoming them. In melanoma, research activity in this area has been focused on understanding resistance to highly selective BRAF inhibition, which induces rapid but usually transient tumor regressions in 70-80 percent of patients.

Studies by Levi Garraway of Dana-Farber Cancer Institute and Roger Lo of University of California Los Angeles have suggested that secondary mutations in BRAF are not a common cause of resistance to BRAF inhibitors, but that multiple other mechanisms seem to be playing a role. Genetic expression studies and functional assays performed on melanoma cell lines before and after treatment with BRAF inhibitors have uncovered mechanisms that reactivate the MAPK pathway: over-expression of the kinase COT, activation of CRAF through multiple mechanisms including NRAS mutation, and MEK1 mutation. In addition, some subsets of melanoma patients have tumors that acquire treatment resistance by RTK (PDGFRbeta or IGF1R) upregulation, turning on a survival pathway that is redundant to the MAPK pathway (Figure 11).

Figure 11: Drug resistance mechanisms

Courtesy of Levi Garraway
In addition, research by Meenhard Herlyn’s laboratory at the Wistar Institute suggests that melanomas contain subpopulations of cells identified by the marker JARID1B that might play an important role in treatment resistance. Combination therapies may be needed to target the different subpopulations. “The goal is to kill all tumor cells and not hope for a bystander effect,” said Dr. Herlyn.

**COMBINATIONS OF MOLECULARLY TARGETED AGENTS**

Greater tumor destruction and preventing or attenuating drug resistance are the goals of combining molecularly targeted agents. For example, combining RAF and MEK inhibitors has shown promising preclinical efficacy, and clinical trials testing this combination of agents are underway. Combining MAPK with PI3K/AKT/mTOR inhibition may be another therapeutic strategy to overcome or prevent acquired resistance to BRAF inhibitors for tumor cells using this redundant survival pathway.

Levi Garraway along with Michael Weber, University of Virginia, and their team are working to identify combinations of molecularly targeted agents that act synergistically on melanoma cell lines. A panel of drugs was examined for the ability to synergize with BRAF inhibition in causing cytotoxicity, and the occurrence of synergy occurred with 36 of the agents, indicating a functional interaction between the targeted signaling pathways. The best combination drug for each melanoma cell line was not determined by BRAF mutational status, which implies that the selection...
of combination therapy agents will have to be part of a more extensive individualized molecular analysis of the tumor.

**COMBINATIONS WITH ANGIGENESIS INHIBITORS**

Greater tumor destruction may result from inhibiting tumor blood supply with bevacizumab and boosting the immune response with ipilimumab. Destruction of the vasculature feeding tumor deposits was observed by Stephen Hodi, Dana Farber Cancer Institute, in biopsies from patient tumors treatment with ipilimumab, indicating this combination could provide additional benefit. Initial clinical tests of this combination in a small number of patients have generated encouraging results, including partial responses and stabilized disease that lasted longer than six months. Additional clinical trials are ongoing to further evaluate this approach.

Anti-angiogenesis treatment may also enhance the effect of chemotherapies in melanoma, as discussed by Svetomir Markovic, Mayo Clinic Rochester. It has been observed in the lab that chemotherapies make tumors more dependent on VEGF for angiogenesis. The addition of bevacizumab to standard chemotherapy drugs (carboplatin plus taxol) led to moderate improvements in overall survival and progression-free survival in Phase II clinical trials. A trial testing nab-paclitaxel, carboplatin, and bevacizumab shows the best improvement in overall survival so far, but a final analysis is forthcoming. These studies demonstrate, said Dr. Markovic, that “there are already treatments on the shelf that could help patients with melanoma.”

These studies demonstrate that there are already treatments on the shelf that could help patients with melanoma.
**COMBINATION IMMUNOTHERAPIES**

The immune response is controlled by a complex system of accelerators and brakes, and manipulating multiple components with immunotherapies may provide additional benefit compared with single agents. One way is to combine checkpoint blockade with potent vaccines that stimulate dendritic cells, such as Drew Pardoll’s TEGVAX technology that can eliminate aggressive melanoma in a mouse model. Another approach is adding immune agonists as described by James Allison of Memorial Sloan-Kettering Cancer Center. Expression of the costimulatory molecule ICOS by T cells has been shown to be associated with biological response to anti-CTLA-4. Using mouse models, blocking CTLA-4 with antibody and immunizing with a tumor cell vaccine expressing ICOS ligand (ICOSL) showed enhanced activity over either agent alone, likely due to increases in T effector cells over regulatory cells and enhanced functionality of T cells. Research is also informing approaches that target multiple immune checkpoints with inhibitors, such as anti-PD-1 plus anti-CTLA-4, because they work at different stages of the immune response. In preclinical studies led by Dr. Allison, blocking these two pathways decreased tumor growth and improved overall survival in a synergistic fashion.

**COMBINATIONS OF MOLECULARLY TARGETED THERAPY AND IMMUNOTHERAPY**

Drugs that kill tumor cells lead to the release of tumor antigens, potentially activating a tumor-directed immune response that may be boosted by the addition of immunotherapies, said James Allison of Memorial Sloan-Kettering Cancer Center. A clinical trial to combine the BRAF-targeting drug vemurafenib with ipilimumab is in the planning stages, and preclinical results are promising. In mouse models, the BRAF inhibitor did not interfere with antitumor effects of CTLA-4 blockade. Preclinical testing is also being
done to combine an HSP90 inhibitor with ipilimumab. Dose and scheduling of drugs is a particularly important component of testing combination therapies. When investigating combinations of molecularly targeted therapies, and immunotherapies in particular, immunosuppressive agents should be given sequentially, whereas agents that do not induce immunosuppression can be given concurrently, as can agents that target different mechanisms that are not likely to interfere with each other.

**RESEARCH CONSIDERATIONS**

More research is needed at both the genomic and proteomic levels to determine the causes of melanoma treatment resistance to inform combination treatment strategies that will prevent and overcome this resistance. Approaches that consider the heterogeneity of tumor cells and the molecular characteristics of each patient’s tumor will be increasingly important because not all cells will be killed in the same way. The combination of therapies acting by different mechanisms, such as molecularly targeted agents or angiogenesis inhibitors and immunotherapies, may provide synergistic effects. However, preclinical efforts are needed to better understand the biological basis for these interactions to develop rational combination trials.

**WHAT THIS RESEARCH MEANS FOR PATIENTS**

Treating patients with more than one agent is expected to improve effectiveness in light of the increasingly evident complexity that researchers are discovering in the network of molecular pathways in melanoma. They are finding that blocking one aspect of a molecular pathway in melanoma often triggers activation of a bypass route, so multiple strategies are needed. In addition, multiple agents may provide added benefit versus using one agent alone. Individual strategies that may be combined in melanoma include agents blocking pro-carcinogenic signaling pathways, agents that boost the anti-tumor immune system response, drugs that block growth of blood vessels that tumors need to survive, and cell toxic chemotherapy drugs. Research is required to determine which combinations make the most sense based on the biological understanding of the cancer and the tumor environment.
Looking Ahead

Significant progress has been made toward a deeper biological understanding of melanoma and in the development of new prevention, diagnostic, and treatment approaches. Investments in identifying new risk genes and prognostic biomarkers for early stage melanoma are starting to bear fruit, but additional investments will be required before they are clinically applicable. For treating metastatic disease, “proof-of-principle” has been established for both molecularly targeted therapies like BRAF inhibitors and immunotherapies like ipilimumab. Yet, much more remains to be done to improve clinical results, including delineating drug resistance mechanisms, identifying biomarkers of patient response, and developing combinatorial regimens.
MRA awards are supporting important lines of research and have catalyzed transformative advances through strategic and accountable investments in research designed to bring near-term benefits to patients and those who are at risk. Recent scientific and clinical successes have set the stage for increased academic and commercial activity in the fight against melanoma. As MRA looks to the future, an updated and revised research agenda will continue MRA’s tradition of strategic investments in research that will address the current and future needs of the field. But no single organization, investigator, or research sector can defeat melanoma alone. The MRA is dedicated to fostering partnerships between all those who share the mission of defeating melanoma. The interactions, discussions, and presentations held at the 2011 MRA Retreat made it evident that robust cross-sector and cross-disciplinary collaborations have, in fact, been fostered by the MRA’s activities. Amazing progress has been made in the field of melanoma recently as a result of partnerships among all those who share the mission to defeat this deadly disease and to continue progress in developing pathways towards a better outlook for patients and those at risk.
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**FOR MORE INFORMATION**, visit the MRA Web site at www.curemelanoma.org. The Web site contains additional information about the MRA research award program and about past retreats.
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