Melanoma from bench to bedside: meeting report from the 6th international melanoma congress

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The 2009 International Melanoma Congress sponsored by the Society for Melanoma Research brought together a large collection of laboratory researchers, students, and clinicians. Presentations were made in numerous areas spanning biological analyses to clinical management of the disease. The meeting was characterized by numerous presentations of provocative, unpublished data, including multiple aspects which impact greatly on our understanding and management of melanoma. This meeting summary has been compiled by the authors in an effort to convey some of the newly presented data despite the fact that a comprehensive treatment of the meeting contents is not feasible. Therefore, the authors apologize to the many speakers and poster presenters whose interesting and important discoveries could not be included here.

**Melanoma pathology**


As a biological phenomenon in melanoma, regression is of considerable importance but nonetheless continues to be very poorly understood. A major problem in the pathology of regression is the ongoing failure to apply standardized and reproducible criteria for its histopathological assessment. Thus, to a significant degree the conflicting data associated with regression and outcome in melanoma can be ascribed to the difficulty and lack of consensus in how regression is evaluated histologically. From this review, it was also concluded that at present there are no objective data that the presence of regression in a primary melanoma is a definite indication for sentinel lymph node biopsy. The scalp constitutes an important site for particular melanocytic lesions such as small cell melanoma in children, both arising de novo and in congenital nevi, blue nevus-like melanomas (malignant blue nevi), and atypical melanocytic nevi that are frequently misclassified as melanoma.

There is ongoing debate about whether a minimal tumor burden may exist in metastatic melanoma, as for example, micrometastases measuring <0.1 or 0.2 mm, and in particular, in sentinel lymph nodes (SLN), which has predictive value for patient management and outcome. On the other hand, there is also evidence that documented metastases of any size may potentially correlate with melanoma progression. A review of the application of SLN biopsy for spitzoid melanocytic lesions has demonstrated SLN deposits in about 50% of patients undergoing this procedure. Interestingly, although tentative, virtually all of the latter patients have failed to show any evidence of disease progression with follow-up.
There has been controversy surrounding the nomenclature of pigmented epithelioid melanocytic neoplasms described as epithelioid blue nevi in the context of Carney complex and the subsequent entity pigmented epithelioid melanocytoma (PEM). However, recent documentation of the mutations in the protein kinase A regulatory subunit 1alpha gene and loss of protein R1alpha expression in the latter lesions provides support for PEM as a distinct entity and a close relationship with epithelioid blue nevi.

Angiogenic melanoma and extravascular migratory metastasis (EVMM) has been the subject of serious research for more than a decade. Accumulating evidence indicates that angiogenesis in melanoma is a prognostic factor, and that extravascular migratory metastasis (EVMM) is an important mechanism of melanoma spread. Recent molecular analysis and gene-expression profiling are beginning to yield new information about the molecular basis of EVMM. Although not studied to any significant degree in melanoma thus far, the potential creation of a ‘premetastatic niche’ by the primary tumor has relevance to melanoma.

Pigmented macular lesions involving chronically sun-exposed skin constitute a challenging spectrum of entities ranging from pigmented actinic keratosis to in situ or thin invasive melanoma. Because of the striking heterogeneity of many lesions, great attention to adequate sampling and careful assessment of margins are needed.

The rates of discordance in the pathological diagnosis of atypical melanocytic lesions are well-established in the literature. However, the basis for this lack of inter-observer agreement has not yet been subjected to rigorous study. One explanation offered for the continued striking increase in incidence of melanoma has been that of ‘diagnostic drift’, or in more precise terms, a diminished threshold for the diagnosis of melanoma by pathologists. A recent study examining intra-observer rates of agreement for interpreting a series of severely atypical melanocytic lesions and thin melanomas with a 20-yr interval has suggested a trend to reduced thresholds for melanoma diagnosis. This outcome was also supported by comparable rates of early melanoma diagnosis among young pathologists evaluating the same study set.

Immunohistochemistry continues to be an important adjunctive technique for confirming melanocytic differentiation and to aid in the distinction of benign lesions from melanoma. In particular, Ki-67, which measures proliferation rate, and markers such as HMB45(gp100) are among the most useful, particularly when assessing topographical expression. For example, benign nevi show reduced expression of the two markers usually in absolute terms but especially with increasing depth, whereas melanomas usually express the two markers throughout. A critical review of the use of biomarkers in melanoma has revealed that Ki-67 is among the very few biomarkers that have been subjected to fairly rigorous investigation. There is accumulating evidence that the routine use of Ki-67 may potentially provide important prognostic information for clinical management of patients.

Among the routine prognostic indices for patients with primary localized melanoma, mitotic rate per mm² has recently been introduced as a major prognostic factor in the new melanoma clinical staging guidelines of the American Joint Committee on Cancer. Finally, with the ultimate goal of obtaining fairly pure or well-characterized melanoma patient populations, a recent study has investigated the reproducibility (inter-observer agreement rates) for particular histomorphological attributes known to correlate with particular genetic alterations, such as BRAF mutations. While the latter studies are encouraging with respect to good inter-observer agreement for many histomorphological parameters, the critical oncogenic alterations equivalent to BRAF are currently not known for a significant proportion of melanomas impairing the utility of genotype–phenotype correlation to improve classification.

Melanoma stem cells

The Stem Cell Satellite symposium commenced with short talk by a leader in the field of reprogramming, Rudolf Jaenisch (Whitehead Institute, MIT), who discussed the barriers and technical challenges of iPS reprogramming. It appears that one of the major barriers to reprogramming is the cellular p53 response pathway. Jaenisch showed that blockade of this pathway, via downregulation of p53, increased the proliferation rate of cells, which in turn dramatically increased the efficiency of reprogramming. Alternatively, overexpression of NANOG could also boost reprogramming efficiency. Jaenisch proposed two different models for acquisition of pluripotency. The deterministic model, which posits that only elite cells (i.e., somatic stem cells) have the capacity to regain pluripotency, versus the stochastic model, which posits that all cells have the potential to generate iPS. Studies from his laboratory favor the stochastic model, indicating that any cell can be reprogrammed to iPS.

The session shifted gears from reprogramming to melanoma and melanoma stem cells with the next speaker, Marcus Rosenberg (Yale Medical School). Mouse melanomas harvested from Tyr::CreER; Braf<sup>CA</sup> Pten<sup>loplox/loplox</sup> mice, in which melanomas spontaneously arise, were analyzed with various stem cell associated markers. Of the many markers analyzed, only CD34, a hematopoietic marker, and p75, a neural crest marker, consistently defined three subpopulations of melanoma cells harboring distinct tumorigenic properties; a CD34<sup>+</sup> subpopulation, which had high colony forming efficiencies and high rates of tumor formation but only gave rise to CD34<sup>+</sup> cells; a p75<sup>+</sup> subpopulation which had
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low colony forming efficiencies and low rates of tumor induction that were highly sensitive to temozolomide and cisplatin, and a CD34+/p75+ subpopulation that gave rise to tumors with a heterogeneous population of CD34 and p75 positive and negative cells. These studies concluded that more than one distinct population of melanoma propagating cells could be isolated from the same tumor and that tumor heterogeneity does not have to be re-established for a cellular subset to form tumors.

While Rosenberg’s mouse model of melanoma development avoids the artificial setting in which human melanomas are typically studied, namely injection of human melanoma cells embedded in Matrigel into immunodeficient mice, the importance of the Matrigel milieu of extracellular matrix molecules, in subcutaneous injection of human melanoma cells provides an artificial advantage to these cells which may not normally produce these factors but that rely on them for tumor propagation. Moreover, the argument was brought forth that the use of NOD/SCID Il2rg−/− mice, which are used by many to assess the tumorigenic capacity of cancer stem cells, provides an advantage to melanoma cells that would otherwise be targeted by the immune system.

Sean Morrison (University of Michigan) agreed that while a greater number of melanoma cells were required to initiate tumors in the NOD/SCID than NOD/SCID Il2rg−/− mice, the former model failed to determine the actual tumorigenic potential of melanoma cells. Indeed, despite their extensive search of over 50 potential melanoma stem cell markers, no single marker or combination of markers, including ABCB5, could distinguish tumorigenic from non-tumorigenic cells in NOD/SCID Il2rg−/− mice. All cells gave rise to tumors, even at the single-unsorted cell level. Morrison’s findings suggest that all melanoma cells have the potential to generate tumors given the appropriate time and environment. Morrison reasoned that while some cancers, including leukemia, fit the hierarchy of the cancer stem cell model, some cancers, melanoma being the prime example, fail to fit this model.

The Congress officially opened with a Keynote Address by David Baltimore (California Institute of Technology). Dr. Baltimore, Nobel Laureate for the discovery of reverse transcriptase, provided a detailed review of his laboratory’s efforts to deploy a strategy of ‘immunologic engineering’ against melanoma. He described three mechanistic opportunities: modulation of dendritic cells, engineering of T cell receptor-modified autologous T cells, and breaking of immunologic tolerance. Of these, his own laboratory efforts have focused on the first two. Baltimore described the design and testing of a novel lentiviral dendritic cell vector which modulates antigen stimulation in a B16 mouse melanoma model by two orders of magnitude. He also described use of recombinant T cell receptor vectors that selectively target tumor associated T cells, to be used in combination with the dendritic cell vector. A clinical trial in man is currently underway (in collaboration with Baltimore’s clinical colleagues at UCLA), and significant responses were described to have occurred in all five patients treated to date. A particularly striking observation was made with respect to the occurrence of ‘mixed responses’ seen in several patients. In one example many metastatic lesions were seen to respond in a patient, whereas one specific lesion had simultaneously progressed despite the immunologic adoptive transfer therapy. The T cell receptor employed in the trial is derived from the laboratory of Dr. Steven Rosenberg (NCI) and is directed against the melanocytic antigen MART1. Biopsy of the isolated ‘progressing’ lesion revealed that this metastasis was essentially devoid of MART1 expression. While loss of antigen expression may thus represent a significant challenge to the success of this approach, the selection for loss of MART1 expression appears to represent a striking ‘proof-of-principle’ supporting the therapeutic strategy’s mechanism of action and in vivo efficacy.

Melanoma immunology

The recent improved understanding of the regulatory pathways of the immune system provides great hope for significant clinical impact. Glenn Dranoff (Dana Farber Cancer Institute) elucidated the important role for granulocyte-macrophage colony stimulating factor (GM-CSF). Through study of GM-CSF deficient mice, GM-CSF was seen to be required for expression of the phosphatidylinerine binding protein milk fat globule epidermal growth factor-8 (MFG-E8) in antigen presenting cells. Interestingly, MFG-E8 within the tumor microenvironment is secreted and contributes to disease progression via αv,β3 integrin signaling. The uptake of apoptotic cells by phagocyte-derived MFG-E8 stimulates Treg through TGFβ, MHC Class II, and CCL22. MFG-E8 limits the efficacy of GM-CSF in vaccination with GM-CSF secreting tumor cells (GVAX) through upregulation of Treg. The use of a dominant negative mutant (RGE) pot-
entiates therapeutic immunity resulting in decreased numbers of Treg. This has specific implications for melanoma, as MFG-E8 expression is linked with progression of the vertical growth phase in primary tumors. Anti MFG-E8 antibodies plus chemotherapy were found to enhance DC activation in tumors by improvement in antigen cross presentation. This proposes blockade of MFG-E8 as a means to combinatorial strategies for melanoma immune therapy.

Continuing the discussion with GM-CSF, Craig Slingluff (University of Virginia) reported on their clinical experience involving vaccination with peptides to tyrosinase, MART-1, gp100, and MAGE family members in the presence of IFA and GM-CSF. The studies included comparisons of 12 peptides to four peptides with local GM-CSF. Monitoring of peripheral blood revealed decreases in peptide specific immune responses in the presence of GM-CSF. This raised the question of the significance of immune monitoring in the peripheral blood versus immune activity occurring at the sites of disease. Biopsies of pre-existing sites of disease following vaccination revealed potent perivascular immune infiltrates with accumulation of both T and B cells. Eosinophils were also present deep within the tumor deposits. At vaccination sites, there was also consistent inflammation with the presence of CD83 dendritic cells. These studies provide further evidence for use of adjuvants in the combination approaches to immune therapy for melanoma and the role of GM-CSF.

A particularly exciting translational clinical investigation currently underway involves B7-H1/PD-1 interactions. B7H1 expression in human cancers is associated with poor prognosis particularly in renal cell cancer and ovarian cancer. Suzanne Topalian (Johns Hopkins School of Medicine) reported early clinical experience with an antibody that blocks PD-1, demonstrating clinical activity in a number of cancers including melanoma. Of particular interest is the detection of persistent PD-1 receptor occupancy following treatment with the antibody. Testing of PD-1 blockade continues.

Adoptive T cell therapy involving the ex vivo expansion of generated antigen-specific T cells offers another promising avenue of investigation for immune therapy. As persistence of transferred cells remains a primary concern, Cassian Yee (Fred Hutchinson Cancer Research Center) described the use of high dose cyclophosphamide as a conditioning regimen for adoptively transferred CD8+ cells against melanosomal differentiation antigens, followed by low dose IL-2. In vivo persistence of cells for up to 10 months was observed, as well as clinical responses including complete response in previously refractory patients. In a separate study, adoptively transferred CD4+ cells also revealed clinical responses. NY-ESO-1 specific CTL infusions induced responses that interestingly also induced immunity to MART-1 and MAGE antigens. The cytokine and chemo-

kine profile significantly influences the helper/trafficking functions of cells.

### Tumor microenvironment

Meenhard Herlyn (Wistar Institute, University of Pennsylvania) presented data that chromatin regulator JARID1B plays a critical role in long-term maintenance of melanoma. Abrogation of JARID1B leads to exhaustion melanoma cells in vitro and in vivo. Only a small subpopulation (~5%) of melanoma cell expressed JARID1B, and these cells were characterized as slowly proliferating cells. As opposed to the cancer stem cell theory, JARID1B did not follow a hierarchical model since JARID1B negative melanoma cells turned into positively expressing (JARID1B) cells, suggesting that environmental cues initiate JARID1B expression. Based on the data, it was proposed that melanoma maintenance is a dynamic process and that the slowly proliferating JARID1B-positive subpopulation is essential for continuous tumor growth.

Mary J.C. Hendrix (Northwestern University) discussed influence of the human embryonic stem cell (hESC) microenvironment on the reprogramming of metastatic phenotype of aggressive melanoma. hESCs express a morphogen Nodal and its natural inhibitor Lefty. Aggressive melanoma cells highly express Nodal, while Lefty is silenced due to DNA methylation. Inhibition of Nodal signaling by hES matrix exposition, antisense morpholinol oligonucleotides or anti-Nodal antibodies revert metastatic melanoma cells toward a more differentiated, less invasive non-tumorigenic phenotype. Hendrix also showed that inhibition of Notch-4 downregulates Nodal expression in melanoma. Their data suggested that the crosstalk between Notch signaling and Nodal signaling contributes melanoma cell plasticity.

Robert S. Kerbel, (Sunnybrook Health Sciences Centre, Toronto, Canada) presented a preclinical model in which melanoma brain metastases occur following the surgical resection of primary tumors. This model was developed using human melanoma xenografts and initially involved the serial selection of aggressive melanoma cell line variants which can easily metastasize to lungs and liver. When mice harboring visceral metastases were treated with doublet low-dose metronomic chemotherapy, survival was enhanced but brain metastases eventually occurred. Cell lines derived from these brain metastases can now ‘spontaneously’ metastasize to the brain in orthotopic tumor models. This model has already facilitated the identification of novel therapeutic targets which can be modulated via small molecule inhibitors to reduce proliferation of metastatic cells.

Sean J. Morrison (University of Michigan) discussed whether melanoma follows a stochastic evolutionary model or a cancer stem cell model. Certain cancers including some germ cell cancers and some leukemias
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are apparently hierarchically organized and only small subpopulations of cells can transfer disease upon transplantation into immunocompromised mice. Only ~1 in a million melanoma cells form tumors in NOD/SCID mice, however one in four can form tumors in a more permissive mouse model (NOD/SCID IL2Rnull mice) when co-injected with Matrigel, suggesting tumorigenic potential is a common attribute of melanoma cells.

Rhoda Alani (Johns Hopkins School of Medicine) discussed melanoma–endothelial cell interaction. Co-culturing metastatic melanoma cells with endothelial cells showed specific patterns of endothelial cell migration. Using a microarray approach, Alani identified neuropilin 2 (NRP-2), a member of the neuropilin family of transmembrane glycoproteins, as a gene that is upregulated in metastatic melanoma cells during interactions with endothelial cells. NRP2 is involved in neuronal cell migration during development and is implicated in tumorigenesis and angiogenesis through its involvement in VEGF signaling. Melanoma-derived NRP2 mediates endothelial cell migration, thus playing an important role in tumor-endothelial cell communication leading to metastasis.

UV and melanoma

Dr. Edward De Fabo (George Washington University) began the session by talking about the roles of specific ultraviolet (UV) radiation wavebands in melanomagenesis. His studies have been focused on UV induction of melanoma in the neonatal hepatocyte growth factor/scatter factor (HGF/SF) transgenic mouse. Dr De Fabo’s previous publications have indicated impressive induction of melanoma by UVB, but no significant role for UVA in melanomagenesis in the albino HGF/SF mouse. In this session, he reported his efforts to extend these studies by examining the possible effects of UVA in pigmented and albino mice with UVB, while UVA showed that thymine dimers were readily generated in both pigmented and albino mice with UVB, while UVA produced oxidative DNA damage only in pigmented and albino animals. However, isolated UVA radiation was only able to produce melanoma in pigmented C57BL/6 mice. Accordingly, he showed that thymine dimers were readily generated in both pigmented and albino mice with UVB, while UVA produced oxidative DNA damage only in pigmented skin. De Fabo concluded, based on his mouse model of human melanoma, that both UVB and UVA can induce melanoma but by two different pathways.

In the next talk, Raza Zaidi (National Cancer Institute) continued on this theme by describing his attempts to uncover in vivo mechanisms by which UV induces melanoma. Zaidi described a tetracycline-inducible, melanocyte-specific GFP reporter mouse that permits the identification and isolation of melanocytes from the mouse following UV exposure. He used this model to identify the in vivo molecular responses of neonatal melanocytes to UVB and UVA both acutely and persistently. In full concordance with known melanomagenic activity in his model, he found that UVB induced a dramatic transient stress response, while reaction to UVA was relatively muted. Zaidi reported that a highly prominent IFN response gene subset was observed after recovery from UVB-induced stress response, associated with a neonate-specific infiltration of macrophages. This macrophage influx was in turn associated with a neonate-specific activation of melanocytic cells, characterized by proliferation and migration toward the epidermis. Zaidi showed that this melanocyte activation was caused by macrophage-derived IFN-γ. He also presented in vivo data indicating that admixed neonatal macrophages can enhance in vivo melanoma growth by suppressing apoptosis. Zaidi suggested that enhanced neonatal susceptibility to UVB-associated melanomagenesis could be explained in part by the action of these macrophages, which could also potentially facilitate future melanomagenesis.

David Fisher (Massachusetts General Hospital) followed with a description of his work on MITF. Control of the mechanisms that underpin responses of the skin to UV involves both cell autonomous and non-cell autonomous events. With respect to pigmentation, much of the regulatory circuitry revolves around MITF, which plays a central role in controlling expression of pigmentation factors. Fisher described the discovery of MITF amplification in advanced melanoma, as well as responses triggered in the skin associated with behavior and homeostatic regulation. Dr. Fisher reviewed his discovery that UV fails to stimulate pigmentation in the absence of functional MC1R in mice, but that pigmentation can be rescued without UV by topical application of forskolin, a cyclic AMP agonist. Chemically induced pigmentation protected against UV-induced squamous tumorigenesis, suggesting a clinical strategy for topical small molecule manipulation of pigmentation. UV also induces expression by keratinocytes of pro-opiomelanocortin (POMC), which can be cleaved to α-MSH, required for tanning. Fisher showed that POMC induction is controlled by, and requires functional p53, which acts as a sensor/effector for UV pigmentation. Pro-opiomelanocortin can also give rise to β-endorphin, which may contribute to human sun-seeking behavior.

Nicholas Hayward (Queensland Institute of Medical Research) discussed his attempts to identify low penetrance melanoma predisposition genes, associated with skin phenotypes and sunlight exposure. Hayward described the exploitation of genome-wide association (GWAS) studies using high-density single nucleotide polymorphism (SNP) arrays to provide unbiased assessment of the association between melanoma susceptibility and the human genome. As a work in progress, Hayward described the evolution of melanoma GWAS from identifying genes already known to be associated with pigmentation phenotypes, to the identification of novel
genes. The original GWAS uncovered risk alleles at classic pigmentation loci, including the MC1R ligand Agouti signaling protein (ASIP) locus. Most recently, low penetrance melanoma risk alleles have been found to mediate their effects through elevating melanocytic nevi numbers. This conclusion is striking because this is perhaps the most convincing melanoma risk factor. Melanoma GWAS studies are now being used to correlate sunlight exposure to pigmentum and nevus risk alleles.

Zalfa Abdel-Malek (University of Cincinnati) talked about her discoveries concerning the role of α-MSH in attenuating UV-mediated DNA damage and inhibiting melanomagenesis. She reported that α-MSH is able to stimulate repair of UV-induced DNA damage and inhibit the generation of reactive oxygen species, mediated through MC1R. Abdel-Malek showed that the α-MSH/MC1R pathway is required for UV-mediated phosphorylation of histone H2AX, essential for maintenance of DNA repair foci at the sites of DNA damage. Forskolin produced the same effect on H2AX as UV, without the requirement for MC1R. Interestingly, UV-induced 8-oxodG production was reduced by α-MSH and blocked by ASIP. Abdel-Malek’s data suggested that α-MSH signaling can reduce oxidative DNA damage by activating nucleotide excision repair, and provided a cogent explanation as to the role of MC1R in melanoma susceptibility.

Melanoma signaling

Ed Harlow (Harvard Medical School, Boston) discussed that because MITF acts in the nucleus in a non-catalytic fashion, it is not considered an easily drug-able protein. But as an activated oncogene critical for the development and survival of melanoma cells, methods of reducing MITF activity would be highly valuable. Cell culture experiments have shown that inhibition of MITF by RNAi sensitizes melanoma cells to the effects of standard chemotherapeutics. They performed a lentiviral RNAi screen for regulators of MITF activity in SKmel5 cells, using the TRPM1 promoter driving a luciferase reporter.

The Harlow group (in collaboration with David Fisher’s lab) found that knockdown of MEK1 and MEK2 led to upregulation of MITF activity, suggesting that, in these cells, phosphorylation by MAPK signaling decreases the half-life of the MITF protein. Interestingly, a similar screen in UACC62 cells identified MAPK1 (aka ERK2) but not MEK1 or MEK2, indicating that the sensitivity point of a common signaling cascade may differ across cell lines. They focused their studies mostly on the genes that led to downregulation of MITF activity, due to their potential therapeutic relevance. Analysis of mRNA levels and promoter-reporter assays showed that the identified candidates exert their effects primarily by decreasing the transcription of MITF. Importantly, inhibition of these genes diminished the viability across several melanoma lines, suggesting conservation of the necessity for MITF and the cellular circuitry regulating its transcription.

E. Elizabeth Patton (Institute for Genetics and Molecular Medicine, MRC Human Genetics Unit, The University of Edinburgh, UK) discussed clues to melanoma development and behavior derived from the nature of melanocytes. Derived from melanocytes which are highly motile neural crest in origin, melanoma has high metastatic potential, and armed with enhanced survival and anti-apoptotic capabilities, melanocytes are naturally resistant to cytotoxic agents. Thus, understanding the ontogeny and biology of the melanocyte – their development from precursor/stem cells, their proliferation, migration, differentiation, interaction with their environment, and death – may be highly informative for new therapeutic approaches to melanoma. The Patton group studies these processes using the zebrafish model system, which allows the visualization of melanocytes in live tissue as well as their progression to melanoma. They have previously generated a BRAFV600E transgenic model of nevi in zebrafish that can progress to melanoma when expressed in a p53 deficient zebrafish. Building on these studies, they showed that animals expressing BRAFV600E in their melanocytes can also generate melanoma in a pten deficient zebrafish (in collaboration with Jeroen den Hertog, the Netherlands) or in a mitf hypomorph background (in collaboration with Jim Lister, USA). Notably, the tumor and molecular pathology of the three BRAFV600E melanomas is distinct, and appears to be determined by the p53, pten or mitf co-operating mutation (Jennifer Richardson and colleagues, unpublished data).

The Patton Group in collaboration with Mike Tyers (University of Edinburgh, UK) have identified a small molecule panel that alters specific aspects of melanocyte biology in zebrafish, and may act as molecular probes to investigate the individual pathways that control melanocyte behavior during development, as well as when the system is perturbed during regeneration and oncogenic transformation. One compound from their screen, tentatively called BIO1E7, appears to selectively kill zebrafish embryonic and adult melanocytes, and may affect melanocyte regenerative processes. Earlier studies from the Patton group suggest that adult zebrafish melanocytes expressing BRAFV600E may be especially sensitive to BIO1E7 treatment (Hironori Ishizaki and colleagues, unpublished data). Current studies using biochemical and genetic approaches may identify the mechanism of action for BIO1E7.

Maria S. Soengas (Spanish National Cancer Research Centre, Madrid, Spain) discussed activation of autophagy pathways in melanoma. It is well known that metastatic melanoma is highly refractory to available radio-, chemo- and immuno-therapy. Therefore, the identification of alternative cell death mechanisms is a priority in this disease. Autophagy is an attractive candidate,
as it holds the potential of promoting autonomous cell killing (e.g., by depletion of key organelles). However, autophagy can also protect tumor cells against a variety of stimuli, including chemotherapeutic drugs which, if any, of these seemingly opposing pro- or anti-tumorigenic functions of autophagy is active in melanoma cells is unknown. Combining electron microscopy and fluorescence-based imaging, the Soengas group identified multiple compounds that led to the formation of autophagosomes either transiently or without affecting melanoma cell viability. However, treatments were also identified to engage autophagy followed by an efficient cell death. These dual autophagy/apoptosis inducers were exemplified by the dsRNA mimic polynosine–polycytidylic acid (pIC). Interestingly, the cytotoxic activity of pIC was strictly dependent on the delivery strategy. Only when targeted to the cytosol (with the polycation polyethyleneimine –PEI), pIC was able to block tumor growth. More importantly, PEI also conferred specificity. Thus, no secondary effects on normal cell compartments were identified upon [pIC]PEI treatments in various animal models. Soengas reported also that their studies have identified the RNA helicase MDA5 as a main sensor of [pIC]. MDA5 served to link dsRNAs to the apoptotic machinery via the protein NOXA. These results provide a proof-of-principle for new tractable points of crosstalk between cytosolic dsRNA helicases, autophagic and apoptotic modulators that could be exploited therapeutically.

Yardena Samuels (National Human Genome Research Institute, NIH) presented data on genome-wide genetic alterations in melanoma. Metastatic melanoma develops through acquired mutations in cancer genes. Comprehensive cancer genome sequencing can identify recurring genetic alterations that may generate fundamentally new, targeted approaches to the diagnosis and treatment of melanoma, enabling a more personalized approach, based on gene mutation profiles of each patient’s tumor.

Matrix Metalloproteinases (MMPs) are proteolytic enzymes that degrade components of extracellular matrix and basement membranes. Matrix metalloproteinases have been associated with cancer metastasis, and small molecule inhibitors of MMPs were tested as potential anticancer agents. However, clinical trials using these inhibitors showed no effect and, occasionally, accelerated tumor growth suggesting that some MMPs can have an anti-tumor role. Samuels systematically addressed these issues by a comprehensive mutational analysis of the MMP gene superfamily in melanoma. The analysis of the MMP gene superfamily identified somatic mutations in 30% of melanomas. Five mutations in one of the most commonly mutated genes, MMP-8, reduced MMP enzyme activity. Expression of wild-type but not mutant MMP-8 in human melanoma cells inhibited growth on soft agar in vitro and tumor formation in vivo, suggesting that wild-type MMP-8 has the ability to inhibit melanoma progression. To further genetically evaluate therapeutically relevant gene families, the Samuel’s group performed a mutational analysis of the Protein Tyrosine Kinase (PTK) gene family in cutaneous metastatic melanoma. This study identified 30 somatic mutations in the kinase domain of 19 PTKs. The entire coding region of these 19 PTKs was further evaluated for somatic mutations in a total of 79 melanoma samples. This analysis revealed novel somatic mutations in ERBB4 in 19% of melanoma cases. To evaluate the functional consequences of ERBB4 mutation, the Samuels group cloned seven mutations that affect conserved residues and are located in close proximity to EGFR mutations described in other tumor types and examined their kinase activity. All seven missense mutations were found to increase ERBB4 intrinsic kinase activity and induce ‘transformed’ activity in NIH 3T3 cells as well as human melanoma cell lines. Melanoma cells expressing mutant ERBB4 exhibited reduced cellular proliferation after shRNA-mediated knockdown of ERBB4 or treatment with the FDA approved pan-ERBB inhibitor lapatinib. Collectively, these results suggest that melanoma cells harboring mutant ERBB4 are ‘oncogenically addicted’ and that melanoma patients harboring ERBB4 mutations may benefit from therapy directed at mutant ERBB4.

Melanoma genomics

The Melanoma Genomics session began with Dr. Michael Stratton of the Sanger Center. Stratton provided initial review of the comprehensive whole-genome sequencing of a single melanoma cell line, Colo829 (derived from a 45-yr-old male's metastatic lesion presumably prior to treatment). Utilizing paired end, Illumina based deep genomic sequencing, Stratton described the 40x genome coverage from the tumor, as well as 35x coverage for the matched normal. Eighty-nine per cent of previously identified mutations in this tumor were found, and 97% of Illumina-identified mutations were confirmed. Overall, the project identified greater than 33 000 somatic substitutions, with nearly 300 occurring within genes, and 182 being non-synonomous (104 silent mutations). Approximately 1000 deletions were identified as well as 37 rearrangements. The overall mutational frequency matched predicted ‘random’ mutational frequency, suggesting that the vast majority are ‘passenger’ mutations rather than ‘drivers.’ No mutations were found in mitochondrial genes. Stratton went on to provide data regarding the clear presence of DNA damage signatures. A vast number of C to T mutations was identified, consistent with a UV induction mechanism. In addition a significant population of C to A mutations was identified, which could be due either to reactive oxygen damage or UV. Stratton explained a strategy utilizing a region on Chromosome 1 with loss of heterozygosities and 4x amplification. This region
revealed that C to T mutations were present in all four copies (suggesting an early mutational event). In contrast, C to A mutations were typically seen in two of the four copies, suggesting they arose later in tumorigenesis, perhaps after metastasis, and thus less likely due to UV, and more likely due to reactive oxygen damage. Stratton also presented evidence that mutational frequency was strikingly lower than random within coding regions, consistent with transcriptional coupled and expression coupled DNA repair. Additionally, mutations were less common in highly expressed genes than weakly expressed genes.

Boris Bastian (University of California at San Francisco) provided a review of the discovery that the GNAQ gene is mutated in a significant fraction of ocular melanomas. This G protein is predicted to activate a number of downstream signaling targets. Bastian went on to report the identification of a second G protein, GNA11, as being mutated in an additional 34% of uveal melanomas, a discovery made in collaboration with Catherine van Raamsdonk (University of British Columbia). The GNA11 and GNAQ mutations are mutually exclusive and together comprise approximately 80% of uveal melanoma cases. Bastian described preliminary data suggesting that the frequency of these two mutated oncogenes is different among liver metastases, compared with primary ocular lesions. Whereas, GNA11 and GNAQ comprised 34 and 46% of ocular melanomas, their frequency was seen to be 59 and 23% respectively among metastases. In contrast to GNAQ which is mutated in approximately 80% of blue nevi, GNA11 is only found in 7% of blue nevi. While these observations may suggest that GNA11 may induce a more aggressive clinical behavior detailed analyses on patient outcome are still pending. The GNA11 mutation occurs in codon Q209, which is the same codon as in GNAQ. Bastian described a clinical strategy to target GNAQ using systemic siRNA delivery for patients with metastatic uveal melanoma.

Lynda Chin (Dana Farber Cancer Institute) spoke in detail about the use of genomics for cancer biomarker discovery. She utilized two murine melanoma models: one highly metastatic and another non-metastatic, to identify expression correlates of metastatic potential. These data were crossed to human genomic gains/losses in primary versus metastatic melanomas to identify candidate metastasis associated biomarkers. Ingenuity (biological pathway software) analysis revealed a disproportionate enrichment among these candidate biomarkers for pathways involved in cancer biology. From these analyses 203 candidate open reading frames were identified and screened by introduction into immortalized human melanocytes for in vitro behavior suggestive of aggressive (invasive) biology. Twenty hits were thus obtained, and one of them, HOXA1, was selected for further analysis. Chin described experiments showing that HOXA1 drives invasion via enhanced TGF-beta signaling. She also discovered that HOXA1 is a biomarker in human breast cancer, and the gene is able to transform mammary epithelial cells.

Levi Garraway (Dana Farber Cancer Institute) described a detailed RNA-based paired-end sequencing project for melanoma. Utilizing a series of early passage melanomas together with matched normal DNA, it was possible to identify somatic mutations as well as a number of translocation events within the tumors. Several fusion transcripts were identified, including one containing RB1-ITM2B, which might potentially create a dominant negative form of the retinoblastoma protein. Read-through transcripts were also found, including one which encompassed the CDK2 gene transcript. Garraway also described separate studies which searched to identify mutations that might confer resistance to MEK inhibitor treatment in melanoma. A series of candidates was identified, based upon in vitro study. One of these resistance alleles was identified in tumor tissue from a resistant tumor isolated from a patient during a clinical trial utilizing the Astrazenica MEK inhibitor, with the resistance allele having been absent from the pretreatment biopsy.

Jeffrey Trent (Translational Genomics Research Institute) described a series of genomics based studies which focus on various skin cancers (including squamous cell carcinoma and melanoma), as well as a broad strategy aimed at ‘personalized medicine’ for cancer patients. This approach is build upon genomics analyses of multiple types which are utilized to make patient-specific predictions. Trent described identification of INPP5A which is lost in squamous cell carcinoma as an early event (also lost in actinic keratoses). He also described the DUSP1 gene in melanoma, whose high expression appears to predict favorable 5 yr prognosis to patients. Trent went on to describe the challenges of current phase 1 clinical trial options for patients, as overall odds for responding to therapy being ~4%. He described construction of a clinical trial at multiple centers which focuses on multiple cancers and multiple molecularly based analyses with the goal of providing ‘molecularly selected drug’ recommendations for patients.

Clues from histomorphology

Dr. Alan Spatz (Jewish General Hospital, McGill University) outlined the importance to develop reliable, quantitative biomarkers for diagnosis and prognostication in melanoma. Dr. Klaus Busam (Memorial Sloan Kettering Cancer Center) reviewed the unique clinical and histopathological features of desmoplastic melanoma, highlighting the implications for a distinct clinical management of this melanoma subtype. Dr. Martin Mihm from the Massachusetts General Hospital presented the concept of the metastatic niche as an important factor determining pattern of metastasis in cancers of various types and the critical role of VEGFR1 signaling.
Transcriptional regulation in melanoma

Ze’ev Ronai (The Burnham Institute, La Jolla, CA, USA) presented new data to support the role of ATF2 in melanoma development. Melanoma development in ATF2 mutant mice (floxed in melanocytes) that were crossed with N-Ras/Ink4a melanoma tumor model was largely inhibited. Analysis of genes that may mediate ATF2 role in melanoma development identified a set of pigmentation genes, including MITF, which are negatively regulated by ATF2 in melanocytes and melanomas. The link between ATF2-MITF and melanoma development is further explored.

In a second set of studies, a novel link between ERK and AKT/PKC signaling was shown. Extending earlier studies from Ze’ev Ronai’s laboratory which established the role of ERK in the upregulation of c-Jun expression and stability, the group now revealed the role of c-Jun in the control of PDK1 transcription. As PDK1 is important in the activation of AKT and PKC signaling, this newly established link underlies the mechanisms accounting for an increase in these pathways by ERK-Jun. Inhibition of melanoma tumorogenesis in a mouse model by attenuated c-Jun was efficiently rescued by re-expression of PDK1, and inhibition of PDK1 by pharmacological inhibitor AR12 effectively blocked melanoma development.

Colin Goding (Ludwig Institute for Cancer Research, University of Oxford, UK) highlighted the rheostat model for Mitf function in melanoma. He showed that up-regulation of Mitf led to a p21-dependent G1 arrest while down-regulation led to a p27-dependent G1 arrest with increased invasiveness. Mitf expression was repressed by Brn-2 that marked a distinct subpopulation of melanoma cells than Mitf. In collaboration with Erik Sahai, real-time intravital imaging of a cell line expressing GFP from the Brn-2 promoter was used to show that within xenograft tumors, invasive cells expressed Brn-2-GFP expression while non-motile cells were pigmented and non-invasive. The presented data suggest a model by which the major driving force behind melanoma metastasis is phenotype-switching induced by changing microenvironment, and suggested that Brn-2 positive cells had properties of melanoma stem cell-like cells.

Michael Green (HHMI/University of Massachusetts Medical School, USA) discussed the role of the secreted protein IGFBP7 in the development and potential treatment of melanoma. IGFBP7 was originally identified by this group in a genome-wide RNA interference screen as one of 17 factors required for an activated BRAF oncogene (BRAFV600E) to induce senescence in melanocytes. Expression of activated BRAF in primary melanocytes leads to synthesis and secretion of IGFBP7, which then acts through an autocrine/paracrine pathway to induce senescence. The induction of senescence involves both inhibition of BRAF–MEK–ERK signaling, which restrains proliferation, and activation of specific senescence-promoting genes. These results reveal a negative feedback loop in which IGFBP7 expression is first activated by BRAF–MEK–ERK signaling and then acts through an autocrine/paracrine pathway to inhibit BRAF–MEK–ERK signaling. He provided evidence that in human melanomas containing an activating BRAF mutation (BRAF-positive melanomas), IGFBP7 may be epigenetically silenced, which appears to be a critical step in melanoma genesis. Restoration of IGFBP7 function by addition of recombinant IGFBP7 (rIGFBP7) induces apoptosis in BRAF-positive human melanoma cell lines, and systemically administered rIGFBP7 markedly suppresses growth of BRAF-positive primary tumors in xenografted mice.

Using a murine model of metastatic melanoma, this group showed that systemic administration of rIGFBP7 markedly suppresses growth of metastatic disease and prolongs survival. Analysis of the NCI60 panel of human cancer cell lines reveals that in addition to melanoma, IGFBP7 induces apoptosis in several other cancer types, in particular colorectal cancer cell lines. In general, IGFBP7 induces apoptosis in human cancer cell lines that harbor an activating mutation in BRAF or RAS, and that are sensitive to chemical inhibition of BRAF–MEK–ERK signaling. Systemically administered rIGFBP7 blocks growth of colorectal tumors containing an activating RAS or BRAF mutation in mouse xenografts.

Estela E. Medrano (Baylor College of Medicine, Houston, TX, USA) presented evidence that the transcriptional co-regulator SKI is involved in melanoma genesis and progression. SKI, a repressor of TGF-β growth inhibitory signals, is prominently detected in human primary and metastatic melanoma tumors regardless of TGF-β levels present in the tumor microenvironment or secreted by the melanoma cells. The Medrano group demonstrated that SKI is required for anchorage-independent growth and for melanoma xenograft growth in vivo. To further evaluate the response to TGF-β this group used RNAi to demonstrate that SKI performs a novel role in that pathway. SKI promotes phosphorylations in the linker region of Smad3 in melanoma cells. These phosphorylations are associated with activation of the oncogenic trait of the TGF-β pathway. They also provided data on how SKI functions as a sensor and modifier of TGF-β signaling by switching protein partners and promoting oncogenic functions. These functions include preventing downregulation of C-Myc and upregulating matrix remodeling proteins and other factors associated with the cancer promotion activities of TGF-β.

Immunologic checkpoints

Improved understanding of regulation of immune checkpoints has provided immense abilities for clinical translation. Thomas Gajewski (University of Chicago) described
the use of human melanoma gene profiling in analysis of vaccination strategies. This work identified three major areas whereby effective anti-tumor immune responses can be inhibited. First, a host response that includes type I interferons is necessary for priming T cells and enabling them to infiltrate tumors. Secondly, some tumors lack chemokines that are necessary to home immune cells to the sites of tumor deposits. Finally, many tumors activate immune suppressive pathways that include PD-L1, indoleamine-2,3-dioxygenase, as well as FoxP3+ Treg. As a result, innate immune activation, immune cell trafficking, and inhibiting immune regulation are all required components of an effective anti-tumor immune response.

Building on mechanisms of immune regulation, Scott Antonia (Moffitt Cancer Center, Tampa, Florida) described the first clinical testing to inhibit the indoleamine-2,3-dioxygenase inhibitory pathway. In a first in man, Phase I trial of 1-MT in patients with solid tumors, a majority of patients experienced decrease in Treg with consequential increase in C-reactive protein. Two patients interestingly developed hypophysitis, both who had received prior immune therapy. This innovative first trial provides tremendous insight for further investigation of this pathway for clinical benefit.

Further expanding insight into the importance of innate immunity, Yang-Xin Fu (University of Chicago) reported that radiation therapy in murine models initiates the production of type I interferons. Use of adenoviral induced expression of IFN-β that mimics radiation therapy expands antigen-specific immune cells. This strongly suggests that ablative radiation can effectively prime the immune system and be an appropriate area to investigate even its systemic effects against cancer.

Finally, Arlene Sharpe (Harvard Medical School) expanded the pre-clinical discussion regarding a key regulator of the balance between T cell activation and immune tolerance. PD-1 is inducibly expressed on T cells and interacts with two known receptors, PD-L1 broadly expressed on hematopoietic and non-hematopoietic cells, and PD-L2 which is inducibly expressed on dendritic cells and macrophages. PD-1 and PD-L1 interactions are critical in priming and expansion of self-reactive T cells, and function to inhibit development of these cells. These interactions also inhibit self-reactive T cells with re-encounter of antigen. In addition, PD-L1 has an important function in controlling Treg. PD-L1 and B7-1 bind at higher affinity than B7-1 with CD28, but less affinity than B7-1 and CTLA-4, and is functionally significant. This sheds critical light into the complexities of immune regulation in the current development of cancer therapeutics.

Animal models of melanoma

Leonard Zon (HHMI Children’s Hospital Boston) began the session on animal models with a summary and update of his use of a fish melanoma screen in the identification of an oncogenic role for the histone methyltransferase SETDB1 in melanogenesis. Zon has developed a novel strategy to test large numbers of genes within intervals associated with copy number gain in human melanoma for their effect on progression. He exploits a strain of zebrafish that develops melanomas as a consequence of the expression of BRAFV600E, the oncogenic mutation found in over half of human melanoma, and p53 deficiency. The screen is based on a mitf rescue. Zon used a mitf-deficient background that suppresses melanomagenesis; therefore, injection of a rescuing mitfa minigene (which also drives expression of candidate cDNAs) into BRAFV600E; p53-deficient; mitfa-deficient mutants results in mosaic rescue of melanocytes and potentially in melanoma. Screening of candidate genes identified only the H3K9 histone methyltransferase SETDB1 gene as able to enhance melanomagenesis. Zon presented data showing that SETDB1 is the major H3K9 histone methyltransferase for tri-methylation in melanoma cells, and that SETDB1 was required for maximum cell proliferation.

In the next talk, Martin McMahon (UCSF/Helen Diller Family Cancer Center) described the new mouse melanoma model he has established in collaboration with Marcus Bosenberg of Yale University. This mouse was designed to express physiological levels of oncogenic BRAFV600E within the endogenous BRAF allele by virtue of activation through Cre recombinase. In these mice, expression of oncogenic BRAFV600E promotes rapid cell proliferation that leads to the development of benign melanocytic lesions. However, McMahon found that these BRAFV600E-induced lesions have an attenuated ability to progress to malignant melanoma unless combined with inactivation of Pten or Ink4a/Arf. Most importantly, the aggressive BRAFV600E; Pten−/− mouse constitutes a highly useful platform for preclinical testing of pharmacological agents that target mutant BRAF, alone or in combination. McMahon has already demonstrated that the combination of inhibitors for BRAF and PI3K can effectively prevent melanoma and lead to its regression; however, tumors invariably recur. This preclinical model has become valuable, because clinical studies of next generation drugs targeting BRAFV600E also demonstrate high response rates, but frequent recurrence.

Lionel Larue (Institut Curie) followed with a description of his own genetically engineered mouse model of metastatic melanoma, developed in collaboration with Dr. Richard Marais of the Institute of Cancer Research in London. Larue discussed the possible roles of NRAS/BRAF, INK4a, PTEN, E-cadherin, and β-catenin in melanocytic transformation. He described the generation of mouse models in which melanocytes harbor mutations for NRAS, PTEN, E-cadherin, and/or β-catenin. Larue described the effects of these activated proteins on
proliferation, immortalization, and migration/invasion in melanomagenesis. Importantly, Larue described his recent efforts to develop a relevant metastatic melanoma model that could be used to study underlying mechanisms.

Glenn Merlino (National Cancer Institute, NIH) then gave the first of two preclinical talks, aimed at exploring the prospects of developing better mouse models for translational studies. The poor predictive power of available preclinical models, including prevailing subcutaneous human xenografts, can be explained in part by their failure to appropriately replicate the clinical setting. In particular, the need to use immunocompromised mice assures the creation of aberrant tumor-host interactions. Merlino detailed his attempts to develop preclinical mouse models that more accurately recapitulate the experience of patients with metastatic disease, focusing on melanoma and non-small cell lung cancer (NSCLC). He described a lentivirus vector that encodes a luciferase–GFP fusion that permits efficient labeling of mouse tumors, and subsequently the ability to track metastatic progression and drug response. Initial success was reported for Lewis Lung Carcinoma (LLC), a well-characterized mouse model for NSCLC that was available as serially passaged tumor tissue (avoiding any in vitro culturing). Lewis Lung Carcinoma tissue was inoculated subcutaneously into syngeneic mice, and after resection, mice were treated in a setting akin to post-surgical first-line adjuvant chemotherapy using clinically relevant agents, and metastasis monitored. Studies are underway to employ this type of technology for melanoma.

Norman Sharpless (University of North Carolina) continued on the theme of testing cancer therapies in genetically engineered melanoma mouse models. Sharpless noted the fact that despite significant advances in the identification and drugging of targets, the number of new anti-cancer compounds making it into standard clinical practice remains frustratingly low. Contributing to this poor showing is the inability of preclinical testing to distinguish effective from ineffec
tive compounds. Sharpless discussed his attempts to address these needs through the establishment of the UNC Mouse Phase I Unit (MP1U), which focuses on the development and use of highly faithful and tractable models of autologous human cancers, and the ability to match improving mouse models to their appropriate human subtype counterparts. Sharpless indicated the ability of the MP1U to perform efficient longitudinal analysis of tumors, with state-of-the-art pharmacologic capabilities and superior compound availability. Dr. Sharpless described his current successful efforts at screening novel anti-cancer agents in mouse melanoma models, alone and in combination, and at identifying those compounds whose activity is significant, specific and with associated with acceptable toxicity.

### Melanoma epidemiology

This session addressed the roles of UV exposure, vitamin D, early detection and genetic susceptibility on melanoma risk and progression. As Maria Teresa Landi (National Cancer Institute) pointed out, melanoma incidence of both thin and thick lesions is increasing in recent years, with a differential pattern by age and sex. Based on the Surveillance Epidemiology and End Results (SEER) registry, incidence in young women (<40 yrs) increased from 5.5 per 100 000 in 1973 to 13.9 in 2004. In women, trunk melanomas show higher incidence in the later birth cohorts (after 1965). Over the same time period, the incidence in young men increased at a much slower rate, from 4.7 to 7.7 per 100 000, with no particular change in patterns of anatomical location. In contrast, incidence rates are much higher in men older than 65, with an approximate 8.8% annual percentage increase since 2003. Peggy Tucker (National Cancer Institute) suggested that the increased risk in younger women may be related to changing habits of sun exposure and increased use of indoor tanning. Approximately 60% of tanning bed users report sunburn, and 32 and 60% of male and female melanoma cases, respectively, used indoor tanning before the age of 30 in a case–control study conducted at two US sites. Though indoor tanning was defined as a class 1 carcinogen from the International Agency for Research on Cancer, regulations for restricting its use are insufficient. As Martin Weinstock (VA Medical Center, Brown University Medical School) emphasized, the US suffers for lack of self-skin examination and of training of primary care doctors in skin examination for early detection of melanoma. In Australia, clinical skin exam <3 yr before noticing mole changes was associated with a 23% reduction of death within 10 yr, and other studies showed improved prognosis after skin examination. Unfortunately, poor compliance in self-skin examination is common (82–91%), particularly in men, although frequency increases with participation of a partner. Although the US Preventive Service Task Force of 2009 has concluded that evidence is insufficient to recommend routine screening for skin cancer, the melanoma epidemiology community is working to impact on these clinical decisions. Another point of debate is whether UV exposure has a beneficial role on melanoma progression. Marianne Berwick (University of New Mexico Health Sciences) presented data suggesting that subjects with signs of solar elastosis around melanoma lesions had reduced progression to metastasis and reduced mortality risk. Moreover, solar elastosis was associated with thinner melanoma lesions, possibly through enhancement of DNA repair or activation of Vitamin D. Epidemiological evidence of a protective effect of Vitamin D against melanoma risk or progression is controversial. Challenges include lack of adequate study designs, concerns about reliability of serum...
measurements, misclassification of sun exposure and vitamin D intake by diet and supplement, and identification of optimal serum levels of vitamin D for cancer prevention. Finally, the discussion moved to genome-wide association studies (GWAS). Tim Bishop (Leeds Institute of Molecular Medicine, Leeds, West Yorkshire) showed results in genetically-enriched melanoma cases from the Melanoma Genetics Consortium (GenoMEL) and from a UK group, which confirmed the association with the melanoma of three pigmentation genes and two regions associated with nevi count. Effects appeared to extend across populations and disease subtypes, and there was no evidence of statistical interaction between the regions. Interestingly, SNPs on chromosome 16 span across a region including three genes: MC1R, FANC, and CDK10. Upon further sequencing of MC1R, it became evident that MC1R was responsible for the association, as SNPs in CDK10 and FANC were no longer significant after adjustment for the additional MC1R variants. This signifies the importance of prior knowledge of melanoma risk factors (like MC1R) even in an apparent ‘agnostic’ approach as GWAS, and highlights the role of epidemiology in further understanding the interplay of genetic risk factors with sun exposure, pigmentation, and nevi formation.

Melanoma clinical translation

This final session presented new clinical results that demonstrated how many of the concepts discussed during the meeting were translated into therapeutic efforts.

Dr. Steven Rosenberg (National Cancer Institute) presented recent work focused on adoptive cellular therapy where three components were key including (1) isolation of tumor infiltrating lymphocytes and ex vivo expansion with tumor antigen; (2) preparation of the patient with lymphodepleting chemotherapy or chemo-radiotherapy to allow engraftment and expansion of the subsequent administered cells; (3) Infusions of ex vivo specific and expanded T cells followed by high dose Interleukin-2. In patients who can respond to known tumor antigens and where T cells can be expanded the approach has led to remarkable response rates with great durability. Upwards of 50–75% of patients have demonstrated RECIST based objective responses with possibly 25–35% being very durable. Additionally, the great majority of patients were refractory to high dose IL-2 alone. In other patients, ex vivo expansion of tumor antigen specific T cells from tumors is not feasible. For these patients and for those with other malignancies, they have pursued the cloning of the TCR from a T cell with MART specificity. The TCR gene has then been expressed in the individual’s own peripheral blood T cells via retroviral transduction. Again the transgenic T cells were infused on the same platform of pre-infusion chemoradiotherapy followed by infusion and then high dose IL-2. Up to 30% of small groups of patients have had objective responses. This opens the ability to use the same reagent for many patients and generate cells like this for other cancers.

Jed Wolchok (Memorial Sloan Kettering Cancer Center) presented an overview of therapy with one of the anti-CTLA4 antibodies, ipilimumab. Following some pre-clinical work and the potential mechanisms of action, he reviewed all the large phase II trials of ipilimumab. While typical RECIST characterized responses are few (<10%) the drug can induce delayed regression after initial early progression in its course. Additionally, some patients demonstrate this delayed response but 1 or 2 tumor sites will progress and can be managed regionally. Examining the long term survival at 2 yr in some studies 40–55% of patients were still alive. Patients with the immune related Response Criteria (irRC) who have progression and then delayed response and those with either delayed or early response followed by isolated progression at single sites that can be managed locally; appear to have a similar outcome to those who show a CR/PR/SD. Wolchok presented his work he is pursuing to identify biomarkers that may allow one to improve the selection of patients for ipilimumab treatment. These may be biomarkers of responses or toxicities. Absolute lymphocyte count (ALC) may be a biomarker for benefit (CR/PR/stable). Similarly circulating CD8+ T cells may be quantitated and appear to be associated with clinical benefit. Additional data were reported on the use of serum level or titer of antibodies directed at a cancer-testis antigen, NY-ESO-1.

Steve Hodi (Dana Farber Cancer Institute) updated attendees on the use of CKIT tyrosine kinase inhibitors in patients with CKIT mutations or amplifications. Previously, it had been demonstrated that CKIT mutations and amplifications were almost entirely observed in those patients with mucosal, or acral melanoma and occasionally in melanomas from heavily and chronically sun exposed areas of the body. Even by selecting this group of patients with acral or lentigious melanoma the frequency of mutations in CKIT was <20% and amplification in 25% of patients (many of which were the mutated population). These numbers held up in his screens of 88 patients. Ultimately, 20 evaluable patients were enrolled into a trial of imatinib. The make up included 10 wildtype and amplified patients and 10 with mutated CKIT. None of the CKIT wildtype and amplified patients responded though two did have stable disease through a few courses. On the other hand, five of 10 of the CKIT mutated patients demonstrated objective responses by RECIST. Trials with Nilotinib (AMN107) a second generation bcr-Abl and CKIT inhibitor and sunitinib, a multitargeted kinase inhibitor both appear to have activity in CKIT mutant patients. These studies are very early. Patient numbers are clearly a challenge due to the rarity of mucosal and acral lentigious melanoma and a frequency of mutations of 15–20%. A question
remains as to how often melanoma from chronic sun damaged skin with contain CKIT mutations, in Hodí’s presentation 2/21 or 10% of those tested demonstrated CKIT mutations. It is anticipated that many investigators will need to collaborate to enroll enough patients to effectively study this disease beyond this initial proof-of-principal.

Lastly, Keith Flaherty (Massachusetts General Hospital) presented results from the phase I/II trial of PLX-4032, a mutant-selective BRAF V600E kinase inhibitor in melanoma patients. The results presented previously at ASCO demonstrated nine of 16 V600E BRAF patients treated with PLX4032 showed a median progression-free survival of 8 months. More recently, there have been an additional 31 patients all with V600E BRAF mutations enrolled and very early results have shown objective responses in nearly 70% of the patients. It was also emphasized that both by clinical improvements and day 15 PET scans, the responses were extremely rapid. Two significant challenges were also pointed out. First, squamous cell carcinomas (generally of the keratoacanthoma type) have developed in up to 25% of patients. Even with close dermatologic evaluation these have developed without antecedent lesions visible. Other toxicity has been quite manageable. Second, and most importantly, is the development of resistance which appears to occur in most patients. This critical challenge may require better scheduling regimens of PLX4032, understanding of what treatment to give patients who progress following PLX4032, and finally what combinations of agents including PLX4032 could be developed to avert resistance and prolong responses. Flaherty described a consortium of multiple melanoma programs across the country with strong centers to develop the next generation of studies driven by scientific advances including the understanding of mechanism of resistance to PLX4032.

In the above-summarized sessions as well as in the well-attended poster session, The Melanoma Congress thus presented new findings from the molecular/genomic to the clinic. Palpable standard-of-care changes in management of melanoma patients are clearly in the winds, from multiple approaches. While it is clear that very major challenges lie ahead, it is also clear that cracks have begun to appear in the previously impenetrable wall surrounding melanoma. A critical mass of outstanding investigators will hopefully continue to build on this momentum. The next International Melanoma Congress Sponsored by the Society for Melanoma Research will be held in Sydney, Australia in November 2010.