

# Meeting Report: Fourth International Congress of the Society for Melanoma Research

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## Abstract

**The 4th international melanoma congress of the Society for Melanoma Research (SMR), organized by Marianne Berwick (University of New Mexico), Paul Chapman (Memorial Sloan-Kettering Cancer Center), Rene Gonzalez (University of Colorado) and Ze'ev Ronai (Burnham Institute), was held at the Marriott Hotel in downtown New York on November 2007. The congress was attended by a record high number of attendees (over 500 delegates) who joined to discuss recent advances in melanoma biology and therapy. About 40% of the participants arrived from 39 countries, a testament to the high impact of this annual gathering on the international melanoma community. Over 120 of the participants were students or postdoctoral fellows, representing a most impressive fraction of young scientists engaged in melanoma research. The meeting consisted of more than 50 plenary and minisymposia presentations, stimulating the exchange of unpublished data and novel ideas, and helping to forge new collaborations that are anticipated to facilitate**

**significant advances in basic, translational and clinical melanoma research. Another major focus of this meeting was over 160 posters, which were heavily attended and provided an effective forum for extensive informal discussions. This report will highlight the major scientific themes and advances of this most successful meeting, and provide a useful perspective on the current state of melanoma research, as well as where the field should be heading.**

doi: 10.1111/j.1755-148X.2007.00437.x

## Day 1/Keynote Address. Speaker: Boris Bastian

Dr. Bastian (University of California at San Francisco, San Francisco, CA) was selected as the keynote speaker for this congress based on his seminal contribution to the melanoma field over the past decade, with important findings highlighting the genetic differences between melanoma subtypes. In his presentation Dr. Bastian showed that melanomas with BRAF mutations differed in their histomorphology from melanomas without such mutations, a finding that could be used to improve the current classification of melanoma to incorporate therapeutically relevant genetic findings. As a follow-up to his prior discovery that KIT is mutant and/or amplified in certain melanoma types, he presented a patient with metastatic mucosal melanoma and a KIT mutation, who showed a major clinical response to imatinib. Dr. Bastian also presented a discovery made in collaboration with Catherine van Raamsdonk and Greg Barsh, that oncogenic mutations in GNAQ are found in over 80% of blue nevi and about 50% of uveal melanomas. GNAQ is a G-protein of the alpha-q family that acts downstream of G-protein coupled receptors. Expression of the mutant protein in immortalized melanocytes was sufficient to induce the transformed phenotypes, with an efficiency comparable to mutant NRAS. GNAQ mutations led to activation of the MAP-kinase pathway and the observation that mutations are mutually exclusive with KIT, BRAF and NRAS indicate that mutant GNAQ may have partially overlapping roles in melanocyte transformation. Dr. Bastian also presented the development of a FISH assay designed to detect chromosomal aberrations in melanocytic tumors. This test was developed based on his group's finding of different patterns of

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chromosomal aberrations between melanoma and nevi. According to Dr. Bastian, the test was designed to improve diagnostic accuracy in melanocytic neoplasms that cannot be unequivocally categorized by current methods. The test will be distributed by Abbott Molecular.

**Day 1/Session 1. Why melanocytes develop into melanoma (early stages in melanoma development). Chair: Dorothy Bennett**

Nearly a decade ago, melanoma was considered a genetic black box. We still lack molecular markers for tumor progression, and the molecular basis underlying the persistent resistance of metastatic melanomas to current anticancer modalities requires better definition. However, we no longer walk in the dark. Comprehensive cytogenetic, mutational and functional analyses in melanoma specimens and cell lines are revealing a complex network of changes in gene expression. Dr. Bennett (St. George's, University of London, UK) reviewed some of what now are considered to be melanoma hallmarks, which appear to develop in a spatio/temporal sequence. A classical example is the BRAF>NRAS>ERK pathway, which is targeted by mutations in early benign melanocytic lesions (nevi). Dysplastic nevi and subsequently primary melanomas with radial or vertical growth patterns (RGP, VGP, respectively) acquire changes in tumor suppressive mechanisms involving p16INK4a/Rb or  $\beta$ -catenin, among others. VGP and metastatic melanomas invariably present with a variety of additional defects in mechanisms of tumor control, including programs of apoptosis and cellular senescence. Thus, *in vivo*, nevus cells are mitotically inactive and show classical markers of premature senescence, including the so-called acidic senescence-associated  $\beta$ -galactosidase activity, SA- $\beta$ -Gal. Melanoma cells, particularly in the VGP and metastatic phases, re-activate telomerase function and acquire additional defects in senescence mediators. Still, it is unclear if nevus cells or cells from *in situ* or non-invasive melanomas (i.e., RGP cells) can be reactivated by appropriate stimuli. To address this question, Dr. Bennett and collaborators biopsied common and dysplastic nevi, as well as RGP and VGP tumors, and determined their clonogenic potential under culture conditions. As expected, nearly all explants from nevi either died or arrested their proliferation after a short-term culture. However, there were exceptions, particularly for dysplastic cases, suggesting that not all cells in nevi are necessarily committed to an irreversible senescent-like state. Proliferative cells could also be isolated from RGP tumors; however, most of these cells were highly unstable in culture, and would senesce or collapse via mitotic catastrophe. These results emphasize the dynamic nature of pigmented lesions, and the fact that tumor suppressive mechanisms acting at early stages of melanoma genesis are potent, but not infallible.

Shin-Ichi Nishikawa (RIKEN, Kobe, Japan) addressed the theme of proliferation, quiescence and differentiation in melanocyte stem cells. Dr. Nishikawa exploited the fact that in mice, at least in mature hair follicles, melanocytes and their progeny (i.e., the differentiated melanocytes) are distributed in geographically distinct compartments. Melanocyte stem cells, present in the bulge region, are maintained in a quiescent state until the hair regeneration cycle is initiated. Proliferating and differentiating melanocytes are located at the bottom of the hair follicle (bulb). The Nishikawa laboratory isolated single cells from the bulge or the hair bulb using as source hair from mice they had previously engineered to express green fluorescent protein (GFP) in the melanocytic compartment (thus allowing for cell sorting). Next, they performed single cell-based cDNA arrays of these different melanocytic populations. A molecular signature for quiescent melanocyte stem cells was found to involve: (i) commitment to quiescence, in part involving NOTCH signaling; (ii) global suppression of housekeeping gene expression associated with RNA polymerase II inhibition; (iii) suppression of Wnt signaling; and (iv) acquisition of a cell autonomous ability to survive in a "niche-independent manner" in the absence of survival mediators such as KIT. Further analyses with proliferating melanocytes are now possible through co-culture with XB2, an immortalized keratinocyte cell line, and specific combinations of SCF and bFGF defined by the Nishikawa group. The question still remains as to how distant or related mouse and human skin compartments are, but this study by Dr. Nishikawa suggests the existence of previously unexplored pathways that may modulate the fate of melanocytic stem cells.

In addition to increased proliferation and blocked differentiation programs, melanomas are notorious for their invasive potential. Since in the human adult skin, mature melanocytes are tightly restricted to the basal layer of the epidermis, it is reasonable to hypothesize that early stages of melanoma development involve the deactivation of potent suppressive mechanism(s) to escape from the control of epidermal keratinocytes. Gavin Robertson (Penn State University, Hershey, PA) presented data showing that the short arm of the chromosome 10 contains one such activity. The starting point of this work was the long known observation that chromosome 10 is frequently lost during melanoma progression. There are well known tumor suppressors identified on the long arm of this chromosome (e.g., PTEN), but not on short arm. The Robertson group used micro-cell fusion to generate cell hybrids to transfer segments of chromosome 10 from a positive donor cell to deficient melanoma cells. Among the hybrid melanoma cells generated, those containing 10p15 were found to have a reduced proliferative capacity when placed in type I collagen. Moreover, xenografts of these cells were significantly less tumorigenic and showed a significantly disorganized ECM (extracellular matrix). Genetic

and functional mapping narrowed the set of candidate genes mapping at 10p15 to KLF6 (Krüppel like factor 6), a gene previously described to control various tumor-associated proteins, including p21, TGF- $\beta$  and E-cadherin. Dr. Robertson showed a progressive loss in expression of KLF6 in RGP and VGP melanoma tumors, and a high frequency of inactivation in metastatic melanoma specimens. By restoring KLF6 levels in deficient melanoma cell lines, Dr. Robertson showed that this protein can restrict activating mutations in the MAPK pathway and modulate survival of melanoma cells that cross the basement membrane and start interacting with, and reorganizing, dermal collagen. These results point to KLF6 as a new tumor suppressor in melanoma.

Scott Lowe (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) presented compelling data that emphasized the power of retroviral-mediated gene transfer to generate mosaic models of cancer in mice. Dr. Lowe presented examples of elegant reconstitution systems where stem cells of hematopoietic or liver nature (to generate specific types of leukemia or hepatocellular carcinoma, respectively) can be transduced by retroviral-mediated gene transfer with candidate oncogenes or tumor suppressors. The retroviruses used can also encode GFP, permitting the sorting of positive cells and fluorescence-based imaging *in vivo*. Genetically engineered progenitor cells are transferred to lethally irradiated mice to repopulate stem cell compartments. Using this approach the Lowe group have identified synergistic interactions between Myc and Bcl-2, p53 or AKT, among others. Dr. Lowe also presented recent work from his group further defining the role of downstream effectors in the mTOR pathway, specifically eIF4E, MNK1/2 or TSC-2 in the development of Myc-driven B-cell lymphomas. Retroviral-mediated gene transfer can also be used to inactivate genes, for example, by transducing short interfering RNAs, or engineered to be activated or inactivated in a conditional manner (e.g., with tetracycline responsive elements). This strategy can be performed for single genes, or in a high throughput manner, using proprietary or commercial shRNA libraries. A key role of Rho-GAP protein (DLC1) was identified in this manner in models of hepatocellular carcinoma. Skin progenitor cells may not be as amenable for whole-body reconstitution assays as hematopoietic or fetal liver precursor cells, but there is still the exciting possibility of genetic engineering artificial human skin for long-term grafting experiments in recipient mice.

### **Day 1/Session 2. Why melanomas are so metastatic (late stages in melanoma development).**

#### **Chair: Menashe Bar-Eli**

Dr. Bar-Eli (MD Anderson Cancer Center, Houston, TX) opened the session by discussing the use of tissue arrays containing nevi, primary and metastatic melanoma for the identification of novel prognostic markers

in melanoma progression. He showed that nuclear-distributed Activator Protein-2 $\alpha$  (AP-2) was high in normal melanocytes and melanocytic nevi and decreased in melanomas in a disease progression manner. In addition, expression of nuclear AP-2 and expression of the Protease-Activated Receptor-1 (PAR-1) protein are inversely correlated during melanoma progression. Dr. Bar-Eli also discussed the tumor promoter activity of platelet activating factor (PAF-1). PAF-1, which is produced in large amounts by the tumor microenvironment, facilitates phosphorylation of the transcription factors CREB and ATF-1, which in turn regulate TGF- $\alpha$ , Bcl2 and other genes involved in melanoma metastasis.

J. William Harbour (Washington School of Medicine, St. Louis, MO) discussed the particular biology of uveal melanomas. These tumors, which are mostly homogeneous, can be classified into two groups. Class1 uveal tumors display mostly a melanocytic morphology, express melanocytic markers and are associated with greater than 95% survival. In contrast, Class2 tumors, which are associated with less than 25% survival, show significant down-regulation of neural crest and melanocytic genes including DCT, SILV and TYR. Class2 tumors display an epithelioid morphology, localization of E-cadherin and  $\beta$ -catenin to the plasma membrane, up-regulation of SPARC and chromosomal alterations. In particular, monosomy of chromosome 3 was found to be significantly associated with metastasis to the liver. Dr. Harbor also reported that Id2, a  $\beta$ -catenin target, is involved in the switch between Class1 and Class2 tumors.

Michael Detmar (ETH Zurich, Switzerland) presented data that melanomas can actively induce the formation of lymphatic vessels, which promotes lymph node metastasis. His recent studies demonstrated that the degree of melanoma lymphangiogenesis can serve as a novel predictor of lymph node metastasis and overall patient survival, and that the extent of lymphatic vessel growth in primary human cutaneous melanomas was the most sensitive parameter for predicting whether these tumors had already metastasized to the sentinel lymph node at the time of surgery. Recently, his group showed - for the first time - that metastatic tumor cells can induce lymphangiogenesis within sentinel lymph nodes, furthering their metastatic spread. Surprisingly, tumor cells induced lymph node lymphangiogenesis already before they metastasized, giving a new twist to the seed-and-soil hypothesis and suggesting that tumors can prepare lymph nodes for their future arrival. This new concept indicates that lymph node lymphangiogenesis represents a novel target for inhibiting and/or imaging melanoma metastasis. Using laser capture microdissection (LCM) and transcriptional profiling of tumor-associated lymphatic vessels, several new activation markers of these vessels were identified.

Don Nguyen (Memorial Sloan-Kettering Cancer Center, New York, NY) described a functional approach

for the selection of metastatic cells *in vivo*, in which he has identified different gene sets that mediate the tissue specific dissemination of cells originally obtained from advanced adenocarcinomas (breast and lung), either as established cell lines or fresh patient pleural effusions. In the context of breast cancer metastasis to the lung, Dr. Nguyen has derived a lung metastasis signature (LMS) that is differentially expressed in a subset of primary tumors from patients that are likely to suffer pulmonary relapse. This clinical validation demonstrates that certain metastatic traits may be already selected in a subset of aggressive primary tumors. Using genetic and pharmacological approaches, Dr. Nguyen showed that the EGF receptor ligand epiregulin, the cyclooxygenase COX-2, and the matrix metalloproteinases 1 and 2 expressed in breast cancer cells collectively facilitate the assembly of new tumor blood vessels, the release of tumor cells into the circulation, and the breaching of lung capillaries by circulating tumor cells to seed pulmonary metastases. These insights reveal how aggressive primary tumorigenic functions can be mechanistically coupled to greater lung metastatic potential, and how such functions may be therapeutically targeted.

### Day 1/Session 3. Why melanomas don't die.

#### Chair: Estela Medrano

Dr. Medrano (Baylor College of Medicine, Houston, TX) opened the session by discussing how chromatin remodeling affects cellular senescence in melanocytic nevi and apoptosis in melanoma. Benign melanocytic nevi are a classical example of oncogene-induced senescence, regulated by the p16INK4a/RB pathway. However, it is unclear what prevents the reversal of senescence since these lesions display a prominent mosaicism in p16INK4a expression. Dr. Medrano reported that histone deacetylase 1 (HDAC1) is required for chromatin remodeling events leading to heterochromatinization and maintenance of the senescence state in nevi and melanocytes, localizing to nuclear foci with RB, SUV39H1 and HP1b. Moreover, overexpression of HDAC1 induced senescence in p16INK4a-deficient melanoma cells. Her data suggest that melanoma regression is achievable by therapies aimed at reactivation of the senescence response. Finally, Dr. Medrano used human melanoma xenografts to demonstrate how SKI, in complexes with HDAC1, curtails the growth inhibitory activity and stimulates the tumor promoting function of TGF- $\beta$ . SKI appears to be critical for melanoma viability as its deficiency prevented melanoma xenograft growth by reactivation of apoptotic pathways.

David Fisher (Dana-Farber Cancer Institute, Boston, MA) presented data on the transcription factor MITF, which plays a fundamental role in melanocyte development, pigmentation, and proliferation/survival. Numerous components of the pigmentation machinery are direct transcriptional targets of MITF, suggesting that it is a central mediator of the pigmentary response. MITF

is also a key target of the MSH signaling pathway. Polymorphisms in the MSH receptor, MC1R, are associated with altered pigmentation, and with non-signaling variants producing the redhair/fairskinned phenotype. Dr. Fisher reviewed evidence that a mouse red-head/fairskinned model (with features of humanized skin) was exquisitely UV sensitive and unable to undergo UV induced pigmentation (tanning). However, topical application of the cAMP agonist forskolin induced significant darkening, which was associated with relative protection against UV-induced skin carcinogenesis. Dr. Fisher also presented a new methodology based upon ChIP-chip and nucleosome positioning microarrays, designed to systematically identify global genomic transcriptional targets of MITF. The approach identifies MITF occupied locations, and overlays this information with nucleosome positioning, to provide chromatin structural data that inform the likely functional importance of MITF at specific locations. Finally, Dr. Fisher presented early clinical data on use of the kinase inhibitor Imatinib in patients with mucosal or acral melanoma. In the study, led by Dr. F. Stephen Hodi, the first KIT mutated melanoma patient to receive imatinib underwent a dramatic clinical and radiographic response at all sites of disease (local and metastatic) within a short time of treatment initiation. Although only a single patient was thus far presented, the striking response suggests that melanoma is indeed vulnerable to oncogene addiction-based therapeutic strategies.

Amato Giaccia (Stanford University, Stanford, CA) discussed the role of bone marrow derived cells (BMDC) in creating a permissive niche for metastatic cell growth. He described the role of Lysyl Oxidase (LOX), a secreted ECM protein involved in cross-linking of collagen and elastin, in tumor cell adhesion and invasiveness. LOX is regulated by HIF1, thought to play an important role in the adaptability of tumor cells to a hypoxic microenvironment and to promote tumor cell invasion and metastasis. Dr. Giaccia described the use of orthotopic tumor transplants, conditioned media and purified proteins to show that hypoxia/HIF1 $\alpha$ -induced LOX is critical to BMDC recruitment and pre-metastatic niche formation in the host organism. His data suggest that LOX may represent an attractive therapeutic target for the treatment and prevention of metastatic disease. Dr. Giaccia also presented data that the prolyl hydroxylase domain protein PHD2 may have tumor suppressor activity, inhibiting tumor growth in a HIF-independent manner; PHD2 may regulate expression of the angiogenic factors Angiogenin and IL-8.

Pablo Lopez Bergami (Burnham Institute, La Jolla, CA) discussed his evidence identifying c-Jun as a critical target of ERK pathway. Constitutively active ERK was shown to increase c-Jun transcription and stability, which are mediated by CREB and GSK3, respectively. The subsequent upregulation of c-Jun increases transcription of target genes, including RACK1 and

PDK1, which in turn positively modulate activation of the PKC, JNK and Akt pathways, suggesting an important role for c-Jun in melanoma.

Barbara Bedogni (Stanford University, Stanford, CA) discussed her work on the interactive roles of Akt and Notch1. The active fragment of the Notch1 receptor (Notch1 intracellular domain, or N<sup>IC</sup>) can have oncogenic activity, and constitutive activation of Notch1 is preferentially expressed in some human melanomas. Moreover, Akt-dependent tumors express high levels of nuclear Notch1-N<sup>IC</sup>. Notch1 activation is associated with activation of the PI(3)K/Akt pathway in melanoma lines. Dr. Bedogni showed that Akt induces Notch1 through NFκB. Moreover, skin hypoxia contributes to Akt-dependent Notch1 activation through HIF1α. Dr. Bedogni concluded that Notch1 is required by Akt to maintain a transformed phenotype in the mild hypoxic environment of the skin, possibly by maintaining melanoma cells in an undifferentiated state.

### Day 2/Session 1. What went wrong in the wiring of melanoma (I): Receptors. Chair: Meenhard Herlyn

Dr. Herlyn (Wistar Institute, Philadelphia, PA) has previously described culture techniques for the generation of melanocytes from human embryonic stem cells (hESCs). This requires growth of hESCs in embryoid bodies and the addition of growth factors Wnt3a, ET3 and bFGF. In this session he described the isolation of melanocyte stem cells from hair follicles and dermis, which have neural stem cell characteristics. When cultured under the appropriate conditions, these cells can give rise to melanocytes, neuronal or smooth muscle cells, chondrocytes or adipocytes. Dr. Herlyn has used these cells in human skin reconstructs to test the effects of melanoma relevant oncogenes. In addition, Dr. Herlyn is analyzing *bona fide* human melanoma specimens to identify melanoma stem cells. His data suggest that melanoma specimens are complex in that they may contain multiple populations of cells with tumor-initiating capacity. Some of the characteristics of these cells are: expression of CD20 (often co-expressed with Mel-CAM) and/or CD133, PKH67 GFP label retention (i.e., slow proliferation) and their presence as a "side population" when stained with Hoechst dyes. Dr. Herlyn is currently subjecting these cells to various analyses to understand their characteristics in more detail and their contribution to melanoma dormancy, invasion, metastasis and drug resistance.

Mary Hendrix (Northwestern University, Chicago, IL) described experiments that implicated the secreted TGF-β superfamily member Nodal as a regulator of the phenotype of aggressive melanoma cells. Under normal developmental circumstances Nodal is a stem cell marker that plays an essential role in mesoderm formation and axis specification. Nodal secretion by melanoma cells was identified using a novel assay for embryonic axis duplication in zebrafish in conjunction with mRNA

analysis using cDNA microarrays. Dr. Hendrix also noted that Nodal expression was elevated in more aggressive melanoma cells compared to their less aggressive counterparts. The activity of tumor specific Nodal expression was promoted by Notch signaling and inhibited by expression of Lefty, produced by embryonic stem cells. This effect may be mediated by methylation of the Nodal promoter. Dr. Hendrix proposed that Nodal expression might be a marker of poor prognosis. Furthermore, inhibition of Nodal expression in melanoma cells led to a reduction in VE-cadherin expression, resumption of melanin biosynthesis and reduced tumor growth, thereby directly linking Nodal expression to the aberrant physiology of the melanoma cell.

Maria Soengas (University of Michigan, Ann Arbor, MI) presented initial data regarding autophagy in melanoma. Cell death can occur by necrosis, apoptosis or through autophagy. Necrosis is not a genetically controlled process, but both apoptosis and autophagy are controlled by elaborate machinery that appears to share some common components. However, whereas apoptosis is solely dedicated to promoting cell death, autophagy can be a protective response that promotes cell survival in the face of nutrient deprivation. Dr. Soengas presented data suggesting that cyclopamine-mediated inhibition of Sonic hedgehog (SHH) signaling through its receptor complex, Patched (PTC) and Smoothed (SMO), leads to activation of a protective autophagy response. This was surprising since this pathway has not previously been linked to autophagy. This response was not dependent on the mutational status of NRAS, BRAF or TP53. However, when cyclopamine was administered in conjunction with either mild hypoxia or serum deprivation, there was extensive cell death. These data indicate that the SHH → PTC → SMO pathway may be a relevant target for melanoma therapy. This is a provocative notion since there are a number of SMO antagonists currently in late-stage preclinical testing or in early clinical trials.

Elena Pasquale (Burnham Institute, La Jolla, CA) described recent work on Ephrin (Eph) receptors and their ligands. In vertebrates, there are 10 EphA (A1-A10) and 6 EphB (B1-B6) receptors. There are also 5 EphA ligands (Ephrin A1-A5) and 3 EphB (B1-B3) ligands. Ephrins and their Eph receptors are implicated in numerous processes such as axon guidance, cell adhesion and cell migration during development, homeostasis and disease. Moreover, Ephrin ligand-receptor interaction can initiate bi-directional signaling such that both the Ephrin ligand and the Eph receptor expressing cells initiate signaling events in response to ligand-receptor engagement. Dr. Pasquale described the interaction between EphA4 and SPAR (Spine associated Rap.GAP). This interaction occurs through the PDZ domain of SPAR and leads to decreased Rap.GTP within the cell. Rap signaling has been implicated in the regulation of cell adhesion and motility through its effects on the affinity

state of integrins. Consequently, Dr. Pasquale claimed that using an anti-EphA4 Fc fragment leads to a decrease in active  $\beta$ 1-integrin that in turn promotes cell migration. By contrast, over-expression of an activated form of Rap1 reverses this phenotype. Finally, Dr. Pasquale noted that SPAR is identical to E6TP1, a protein that binds to the E6 oncoprotein of human papilloma virus and is subsequently proteolyzed. This observation potentially links SPAR/E6TP1 to the pathogenesis of human cervical cancer. In separate experiments, Dr. Pasquale described that EphB4 is expressed in a wide variety of melanomas and that expression correlates with invasion and metastasis. Dr. Pasquale described the generation of a peptide that competes for the EphB4 ligand-binding site, and does so at remarkably high affinity. Peptide-mediated blockade of the interaction between EphB4 and its natural ligands may be an effective strategy to inhibit melanoma cell invasion and metastasis.

### **Day 2/Session 2. What went wrong in the wiring of melanoma (II): Kinases. Chair: Martin McMahon**

Dr. McMahon (UCSF Comprehensive Cancer Center, San Francisco, CA) opened the session by discussing the macro and micro effects of the BRAF<sup>V600E</sup> mutation on the biology of melanocytes. When expressed at low levels BRAF<sup>V600E</sup> induces cell cycle progression; however, even at moderate levels it induces growth arrest by inducing the expression of cell cycle inhibitors p21Cip1, p16Ink4a and p15Ink4b. Dr. McMahon reported the generation of mice carrying a Cre-activated BRAF<sup>V600E</sup> allele. Mating these animals with mice harboring a 4-hydroxytamoxifen-inducible Cre recombinase-estrogen receptor fusion transgene under the control of the tyrosinase promoter resulted in melanocytic hyperplasias after tamoxifen treatment. Interestingly, these pigmented lesions did not show evidence of melanoma progression unless combined with loss of Pten function, in which case the lesions progressed rapidly to malignant melanoma with evidence of invasion and distant metastasis. Marcus Bosenberg (University of Vermont, Burlington, VT) then continued the discussion of these genetically engineered mice with a focus on melanoma progression and metastasis. Animals with an activated BRAF<sup>V600E</sup> allele on an Ink4a/Arf-deficient background rarely displayed melanoma lesions. However, the rare tumors that did appear showed rapid melanoma progression. In contrast, when activated BRAF<sup>V600E</sup> was combined with loss of three tumor suppressor genes: Pten, Ink4a and Arf, the animals displayed robust formation of primary cutaneous malignant melanomas with dramatic evidence of lymph node invasion and evidence of metastasis to several organs.

Richard Marais (Institute of Cancer Research, London, UK) described the generation of a mouse model that was quite similar to the one described by Drs. McMahon and Bosenberg. However, in this case,

activation of BRAF<sup>V600E</sup> expression during embryogenesis led to a multiplicity of developmental defects that include macroencephaly, surface brain nodules, heart and eye defects that led to lethality. Cells isolated from these mice proliferated in tissue culture and generated tumors as xenografts. Genotyping suggested that the transformed cells were melanoblasts. Using a conditional Cre allele generated by Dr. Lionel LaRue, Dr. Marais described the effects of BRAF<sup>V600E</sup> expression in adult melanocytes that bore strong similarity to the data of Drs. McMahon and Bosenberg, with the exception that Dr. Marais saw evidence of frank melanoma progression at late times after BRAF<sup>V600E</sup> expression. Finally, Dr. Marais discussed that BRAF<sup>V600E</sup> regulates MITF expression through effects on MEK/ERK signaling and the transcription factor BRN2. Moreover, BRAF<sup>V600E</sup> acts through MITF to promote expression of components of the cell cycle machinery leading to melanoma cell proliferation.

Frank McCormick (UCSF Comprehensive Cancer Center, San Francisco, CA) discussed the success and failure of therapies targeted to oncogenes. Dr. McCormick described how drug resistance, activation of critical signaling pathways and tumor heterogeneity affect survival benefits of such therapies. In particular, it was demonstrated that single molecule agents are not effective in treating malignant melanoma. A paradigm of such failure is the multi-kinase inhibitor Sorafenib, which paradoxically is active in renal cell and hepatocellular carcinomas. Once more, melanoma shows its ability to escape from promising therapies.

### **Day 2/Minisymposia Session A. Immunobiology. Chair: Soldano Ferrone**

Dr. Ferrone (University of Pittsburgh, Pittsburgh, PA) began by first announcing that he was chairing the session in place of Dr. Alan Houghton, to whom he wished a speedy recovery. Dr. Ferrone's research focus has been on immunity to the high molecular weight melanoma-associated antigen (HMW-MAA). This antigen is present not only on melanoma cells, but also on glioma, chordoma, chondrosarcoma and acute lymphoblastic leukemia. Immunity to this antigen is also thought to represent a means of angiogenic targeting. Dr. Ferrone presented data from a prior clinical trial using an anti-idiotypic monoclonal antibody in patients with melanoma. Patients who developed an antibody response to HMW-MAA as a result of the anti-idiotypic antibody had better overall survival compared with historical controls. Dr. Ferrone is now using the antibody in a SCID mouse model using breast cancer cells thought to have stem cell-like properties. Evidence for an anti-angiogenic effect was presented.

Jedd Wolchok (Memorial Sloan-Kettering, New York, NY) presented data on the use of immunomodulatory antibodies as therapies for melanoma in both preclinical and clinical studies. The most advanced approach is the

use of anti-CTLA-4 monoclonal antibodies, currently in phase III studies by Medarex/BMS as well as Pfizer. There have been documented clinical responses as well as reversible immune-related adverse events, serving as proof of principle for the mechanism. Data showing the spectrum of kinetics of clinical response as well as induction and augmentation of immune responses to cancer-testis antigens was also presented. This approach has significant potential in the therapy of metastatic melanoma patients. Preclinical studies of antibodies to GITR and OX40 in the B16 mouse model have demonstrated significant biologic activity.

Raphael Clynes (Columbia University, New York, NY) gave an insightful presentation regarding the importance of Fc receptors in tumor immunity. Dr. Clynes' prior work has clearly shown the necessity of Fc receptors in mediating the anti-tumor effects of rituximab and trastuzumab in mouse models. He has also worked extensively using TA99, a monoclonal antibody recognizing TRP-1, and showed that Fc receptors are required for the tumor protection and autoimmune hypopigmentation mediated by TA99 in the B16 model. One hypothesis for the efficacy of monoclonal antibodies is that they may allow for cross-presentation of their target antigens through Fc receptor binding on antigen presenting cells. This has been evaluated in mice using ovalbumin and now in human breast cancer patients who had received trastuzumab in the past. A subset of patients treated with trastuzumab develops antibodies to her-2/neu, and these patients appear to have a superior clinical outcome to similarly staged and treated patients who did not develop antibodies.

Cassian Yee (Fred Hutchinson Cancer Research Center, Seattle, WA) presented his group's data on the use of adoptive transfer of T cell clones in patients with metastatic melanoma. The approach Dr. Yee is using involves *ex vivo* expansion of CD8+ and CD4+ clones and transfer into patients treated with fludarabine for lymphodepletion. Serum IL-7 and IL-15 levels increase approximately one week following fludarabine, suggesting the induction of homeostatic proliferation. The most recent trial has used CD4+ T cell clones directed to epitopes on tyrosinase and NY-ESO-1. Early data show several clinical responses of this approach.

The final presentation of the session was by Kate Dadachova (Albert Einstein College of Medicine, Bronx, NY). Dr. Dadachova has been working with an anti-melanin monoclonal antibody that was originally raised to fungal melanin (*Cryptococcus*) but also recognizes melanoma cells. A phase I trial has been initiated in Israel investigating the effects of a radio-labeled form of the antibody in melanoma patients.

#### **Day 2/Minisymposia Session B. Gene expression/micro RNA. Chair: Jeffrey Trent**

This session chaired by Dr. Trent (TGen, Phoenix, Arizona) began with a talk by Dr. Kevin Brown (TGen,

Phoenix Arizona), who discussed the use of mining genomic and phenotypic data to identify novel melanoma genes. For such studies, they are using a panel of early passage primary melanomas treated or not with the chemotherapeutic agent temozolomide. Dr. Aleksandar Sekulic (TGen, Phoenix, Arizona) discussed the use of high-resolution oligo-CGH for the detection of subgenomic modifications in melanocytic nevi and melanomas. Interestingly, this group reported that benign nevi already show small deletions and copy number variations. Dr. Nick Hayward (Queensland Institute for Medical Research, Herston, Australia) reported the use of genomic copy number with global mRNA and microRNA for cataloging genes in melanoma development. Dr. Alain Spatz (Institut Gustave Roussy, Villejuif, France) described the identification of gene-profile signatures for human primary malignant melanoma associated with metastasis to distant sites and poor prognosis. DNA replication and DNA repair pathways were among the most significant pathways associated with progression to metastasis. The overexpression of DNA repair genes could mediate the notorious resistance of metastatic melanoma tumors to current therapeutical modalities. Dr. Daniel Peeper (The Netherland Cancer Institute, Amsterdam, The Netherlands) described a study aimed at defining mediators of BRAF<sup>V600E</sup>-induced senescence. Microarray analysis identified IL-6 as a major outlier. Dr. Peeper also found that C/EBP $\beta$ , a downstream target of Ras signaling, is activated by BRAF<sup>V600E</sup>. Functional studies demonstrated that induction of senescence by IL-6 is cell-autonomous. This group also reported that C/EBP $\beta$  is recruited to the IL-6 promoter, thus these proteins may control sets of overlapping genes in oncogene-induced senescence. Together, the studies discussed in this session may lead to the discovery of new prognostic markers and therapies for the treatment of melanoma.

#### **Day 2/Minisymposia Session C. Epidemiology and prevention of melanoma. Chair: Richard Gallagher**

Dr. Gallagher (BC Cancer Research Centre, Vancouver, Canada) reported that sunbed use is increasing in most western countries particularly among young people, and concerns have arisen about their effect on melanoma risk. Because most studies to date have been small, risk estimates have been unstable with wide confidence intervals. Several recent meta-analyses have been conducted, and show a modest elevated risk for 'ever' versus 'never' use of sunbeds (RR=1.15; 95% CI= 1.00-1.31). Control for concomitant sun exposure produces little change in the point estimate of risk. First exposure 'in youth' (earlier than age 35) appears to further increase risk (RR=1.75; 95% CI=1.35-2.26), and risk appeared to be higher with exposures 'distant in time' by comparison with 'recent' exposures. Dr. Gallagher concluded that findings of increased risk with early life use, coupled with what appears to be a greater risk with increased lag time, parallel trends seen with

exposure to solar UV radiation and suggest that an appropriate public health policy might be to more severely restrict use of sunbeds by those under the age of 18.

David Whiteman (Queensland Institute of Medical Research, Brisbane, Australia) reviewed the accumulating evidence from epidemiologic studies that cutaneous melanomas arise through different causal pathways. In the realm of descriptive epidemiology, it is now clear that the patterns of age-specific incidence of melanoma at different anatomical sites behave in a consistent manner in all fair-skinned populations. Melanomas arising on intermittently exposed body sites are significantly more common among younger and middle-aged adults, whereas melanomas of the head and neck are most common among older people. Dr. Whiteman reviewed data from analytical epidemiologic studies that have generally supported these findings, and have shown that people who develop melanoma of the head and neck tend to have fewer nevi, greater lifetime exposure to sunlight and more evidence of chronic solar damage than those who develop melanoma of the trunk. Most recently, molecular epidemiologic studies have explored this issue by comparing the characteristics of melanomas harboring BRAF mutations compared to those with wild-type BRAF. BRAF mutant melanomas tend to arise on the trunk and occur among younger people with many nevi. Dr. Whiteman concluded that taken together, these epidemiologic findings accord with recent molecular and animal studies that melanomas arise through multiple causal pathways.

Nancy Thomas (University of North Carolina, Chapel Hill, NC) discussed how the Genes, Environment and Melanoma (GEM) consortium used mutational analysis of melanoma as a tool for investigating causal pathways in melanoma. She discussed their results that melanomas with BRAF mutations and melanomas with NRAS mutations were associated with different risk factors. Compared to patients with neither mutation in their melanoma, patients with BRAF-mutant melanomas tended to be younger, to have a lot of moles, and to have lived in a sunny climate between ages 0 and 25. Patients with NRAS-mutant melanomas also tended to have increased numbers of moles, but were typically older and tended to have lived in a sunny climate after age 50. Dr. Thomas concluded that both early and late life sun exposure are important to risk, albeit for different tumor genotypes. Thomas also discussed that theories of melanoma heterogeneity were supported by unique incidence and age distribution patterns by site of melanoma examined using the large Surveillance, Epidemiology, and End Results Program (SEER-17) database.

Mark Elwood (BC Cancer Research Centre, Vancouver, Canada) presented a summary of an analysis of melanoma occurrence in Queensland, Australia, which has the highest incidence of melanoma in the world. A unique series of detailed studies in this community

makes it possible to describe the occurrence, diagnosis, primary management and outcomes for melanoma, as well as the knowledge, attitudes and screening and preventive practices of the population. Knowledge about skin cancer is very high, and high proportions of the population practice skin self-examination, and/or have a regular skin screening from a physician; yet most melanomas are detected as incidental findings. Dr. Elwood reported that greater awareness and surveillance has given a great increase in the diagnosis of thin melanomas; however, this trend has not been followed by any clear reduction in the incidence rate of more deeply invasive melanomas. Dr. Elwood suggested that this finding has many possible implications, one being how often thin melanoma is not, biologically, destined to progress to thicker and metastatic melanoma. This is an issue that cannot be directly studied in humans and must be assessed from clinical and epidemiological data.

Marianne Berwick (University of New Mexico Cancer Center, Albuquerque, NM) talked about gene-environment interaction in melanoma, focusing on the population-based evaluation of genetic variants in the major familial melanoma gene, CDKN2A, its prevalence and its interaction with sun exposure. Dr. Berwick presented data indicating that 1.2% of 2,424 melanoma patients from the general population from the US, Canada, Italy and Australia had a functional mutation in this gene and that 3.2% of 1,189 patients with multiple primary melanomas from the same population registries in the 4 countries also had a functional mutation in this gene. Therefore, an almost 5-fold increased risk for developing melanoma with a CDKN2A mutation was found, lower than the 10-fold increased risk anticipated based on previous, family-based studies. In the same study, the GEM study group found that excessive intermittent sun exposure over a lifetime increases risk about 1.6-fold, similar to previously reported meta-analyses. Synergistic interactions between sun exposure and mutations in CDKN2A were detected in a "case-only" design, although not in a case-control study design. Dr. Berwick finished by asserting that all subjects, not only mutation carriers, should practice "safe sun" exposure - that is, avoiding intense intermittent sun exposure during their lifetime.

## **Day 2/Minisymposia Session D. Clinical updates.**

### **Chair: Lynn Schuchter**

The session began with Dr. Schuchter (University of Pennsylvania, Philadelphia, PA), who discussed 2010 as a target date for improving survival in patients with melanoma. In this session, Dr. Schuchter explored the unique challenges in melanoma treatment and the current obstacles to achieving the 2010 goal. Ongoing phase III clinical trials were reviewed, and it is not clear that existing phase III clinical trials are on target to improve overall survival in patients with metastatic,



stage IV melanoma. Dr. Schuchter raised a number of key points during this presentation. When planning for clinical trials, particularly with targeted agents, it is critical to recognize the heterogeneity of melanoma with respect to molecular as well as clinical characteristics. Clinical trials will need to have sufficient power to take into account this heterogeneity; therefore much larger phase II and phase III studies will be needed. Optimally defined patient populations are key, especially for targeted therapies. There should be mandatory collection of tumor blocks on all patients enrolled on clinical trials, a practice that is critical to understanding the relationship between various molecular alterations and response/resistance to therapy. Well defined, consistent metrics of success of phase II studies (i.e., PFS) are needed to appropriately select treatment regimens for the next phase III studies. It will be critically important to coordinate ongoing phase II studies so that we are poised to test the best agents in the properly selected patients in phase III studies. Future efforts will require a highly organized effort across clinical centers with close collaboration with scientists.

Keith Flaherty (University of Pennsylvania, Philadelphia, PA) presented an update on the clinical development of RAF and MEK inhibitors. Unregulated intracellular signaling in the MAP kinase and PI3 kinase pathways appears to be central to melanomagenesis. The rationale for targeting MAPK pathway was presented. Currently there are three drugs in clinical development that target BRAF. Sorafenib, the least selective agent, was the first available RAF kinase inhibitor that was available in 2002 when BRAF mutations were first identified in melanoma. Clinical trials with sorafenib, 400 mg given orally twice a day, as a single agent showed little activity in more than 50 patients with metastatic melanoma. However, when combined with chemotherapy sorafenib appears to have greater clinical activity, which has led to three randomized clinical trials. Agents undergoing testing in combination with sorafenib include dacarbazine, temozolomide, paclitaxel and carboplatin. More potent and selective RAF inhibitors (RAF-265) and PLX4032 are currently being evaluated in phase I/II clinical trials. These agents also appear to have greater selectivity against BRAF and NRAS mutant lines *in vitro*. In the second part of his presentation, Dr. Flaherty discussed whether MEK inhibition offered an advantage over RAF inhibitors. Two MEK inhibitors are currently being evaluated in patients with melanoma, PD 0325901 and AZ26244.

Barry Allen (University of New South Wales, Sydney, Australia) presented the results of a preliminary study on the use of alpha-emitting radioisotope Bi-213 chelated to the 9.2.27 monoclonal antibody, which is highly specific for the melanoma-associated chondroitin sulfate proteoglycan NG2, to form an immunoconjugate. Forty patients with stage IV melanoma were treated. There was no evidence of toxicity and responses were seen,

with 12% of patients experiencing regression of subcutaneous lesions. The mechanism of the anti-tumor response is not clear. Dr. Allen suggested that the responses seen were not related to targeted alpha therapy. Rather, Dr. Allen's hypothesis is that observed regression of tumors was mediated by a mechanism called tumor antivasculature therapy (TAVAT). This effect depends on the vascular permeability of tumor capillaries, the expression of targeted receptors by capillary pericytes and contiguous melanoma cells, and on the short range and high-energy transfer of alpha radiation.

Rhoda Alani (Johns Hopkins University School of Medicine, Baltimore, MD) and her colleagues have taken a reductionist view of tumor metastasis by evaluating direct interactions between melanoma cells and endothelial cells in order to better understand cell-cell communications that take place during metastatic events. Dr. Alani has identified neuropilin 2 (NRP-2), a member of the neuropilin family of transmembrane glycoproteins, as a gene that is upregulated in 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 the VEGF signaling pathways. This group has found that inhibition of NRP-2-related signaling using blocking antibodies has yielded a greater than 90% reduction in proliferation of melanoma cells. Random melanoma cell migration and melanoma invasion is also reduced in the presence of NRP-2 blocking antibodies. Interestingly, siRNA-mediated silencing of NRP-2 does not significantly impede melanoma cell proliferation, suggesting a novel titration mechanism involving VEGF and VEGF receptors *in vivo*. Preclinical studies already underway in a mouse model system will help further define the role of NRP-2 in melanoma metastasis *in vivo*. Since humanized monoclonal antibodies to NRP-2 are currently under development as anti-angiogenic therapies, Dr. Alani has proposed that such novel therapies will be specifically useful as targeted therapies for melanoma given their anti-tumoral effects in these cancers, and she expects that phase I clinical trials of these targeted agents will include significant numbers of melanoma patients at the outset.

Kowichi Jimbow (Sapporo Medical University School of Medicine, Sapporo, Japan) and his colleagues reported on their new approach, melanogenesis-targeted therapy, drug delivery system and chemo-thermo-immunotherapy. This is based upon their previous work that showed that the N-propionyl derivative of cysteamine-phenol is a good tyrosinase substrate, and is selectively incorporated into melanoma cells. Encouraging anti-melanoma effects have been seen in patients with stage III and Stage IV melanoma.

Paul Chapman (Memorial Sloan-Kettering Cancer Center, New York, NY) presented very early results from a phase II study of neoadjuvant temozolomide in patients with melanoma. The overall goal of this ongoing study

was to identify tumor characteristics associated with response to Temozolomide. To be eligible for this study, patients were required to have palpable disease and be candidates for complete resection of melanoma. Treatment consisted of a pretreatment tumor biopsy, followed by extended dosing of Temozolomide at 75 mg/m<sup>2</sup> daily for 6 weeks followed by 2 weeks off. Patients were treated until best response and then surgery was performed. To date, 13 patients have been treated, with 2 complete responses and 1 partial response. Tumor biopsies are being evaluated for MGMT promoter methylation. Importantly, as presented by Dr. Chapman, this neoadjuvant approach offers a unique opportunity to study tumor tissue before and after treatment.

Striking at this session was the wide gap between ongoing basic science of melanoma and clinical trials. The need for communication between basic scientists and clinicians has never been more critical.

### **Day 2/Session 3. What went wrong in the wiring of melanoma (III): Transcription factors. Chair: Colin Goding**

Dr. Goding (Marie Curie Research Institute, Oxted, UK) highlighted the regulation of the Microphthalmia-associated transcription factor MITF as a key factor in melanoma proliferation and metastasis. Previous work from this group demonstrated how MITF is necessary to maintain expression of Dia1, a diaphanous-related formin that coordinates the actin and microtubule networks at the cell periphery and indirectly controls the stability of the p27 cyclin-dependent kinase inhibitor. Thus cells with low MITF exhibit a p27-dependent G1 arrest and have high invasive potential. Since MITF is highly regulated at the transcriptional and post-transcriptional levels, the data suggest a model in which melanomas contain MITF-positive proliferating/differentiating cells, and an invasive MITF-negative population, and that switching between these two populations would be regulated by the microenvironment. To identify the MITF-negative cohort, they first identified the BRAF and  $\beta$ -catenin regulated Brn2 transcription factor as a negative regulator of the MITF promoter. Then, Dr. Goding showed that areas of tumors that would normally be classified as double positive for MITF and Brn2 by conventional immunohistochemistry of adjacent sections, in fact contained two distinct cohorts as determined using double immunofluorescence. The data support the idea that the success of any therapy must take into account the heterogeneous nature of melanoma at the cellular level. Dr. Goding's data showing that Brn2 represses MITF expression was in contrast to those of Dr. Marais, who showed that Brn2 could activate MITF expression; it was concluded that Brn2 activity is likely cell line dependent.

Lionel Larue (Institut Curie, Orsay, France) discussed the function of an oncogenic mutant form of  $\beta$ -catenin in melanocytes *in vivo*, as well as the function of its target Brn2. Importantly,  $\beta$ -catenin was found to induce

melanocyte immortalization by directly repressing p16INK4a expression, and to cooperate with NRAS to produce melanoma and metastases. Threonine and serine residues are highly conserved in the pou domain of Brn2. In order to understand the function of such residues, transgenic mice expressing wildtype Brn2 or mutationally inactivated Brn2 (Brn2AA) were generated and characterized. Dr. Larue reported that wildtype Brn2, but not Brn2AA, induced melan-a cell proliferation *in vitro*. Brn2 wildtype mice exhibited a slight hyperpigmentation of the extremities, while Brn2AA mice showed a white belly spot and hypopigmented tail and paws. Brn2AA was found to be dominant over wildtype Brn2. Interestingly, both Brn2AA and wildtype Brn2 were able to repress the expression of MITF. However, Brn2 activated Pax3 expression while Brn2AA repressed Pax3 expression. Notably, transgenic mice expressing either wild-type Brn2 or the Brn2AA mutant failed to develop melanoma.

Gerard Evan (UCSF Comprehensive Cancer Center, San Francisco, CA) reviewed the prospects for exploiting the potential of the transcription factors Myc and p53 as therapeutic targets. Using an experimental approach he calls "kinetic genetics", Dr. Evan can activate estrogen receptor (ER) fusions of these transcription factors *in vivo* by exposing appropriately genetically engineered mice to tamoxifen. This effect is reversible, as withdrawal of tamoxifen inhibits the fusion protein. For example, Dr. Evan showed that re-expression of p53 was lethal to p53 deficient tumors, which succumbed to massive apoptosis. Tetracycline inducible expression of OmoMyc, a potent dominant negative leucine zipper able to poison partner interactions, also eradicated tumor tissue, even in mouse cancer models not driven by Myc. Surprisingly, these Myc impaired mice were relatively healthy, a positive sign when thinking about possible clinical value. When considering p53 and Myc as therapeutic targets, Dr. Evan stressed that it will be important to determine if these tumors recur after "treatment", and if so will they then respond to a second round.

Gianluca Civenni (University of Zurich, Zurich, Switzerland) presented evidence that metastatic human melanomas and melanoma cell lines contain a cellular subpopulation resembling neural crest stem cells (i.e., p75+). These p75+ cells were reported to be able to form tumor spheres, and self-renew and form tumors upon serial transplantation resembling the tumor of origin, while their p75- counterpart cannot. The melanoma stem cell is an extremely exciting concept, but more data will be needed to prove its existence and the value its treatment may bring to the clinic.

Therese Becker (Westmead Institute for Cancer Research, Sydney, Australia) described her use of a yeast-two-hybrid screen to identify the chromatin-remodeling factor BRG1 as a novel p16INK4a binding partner. Becker found that the endogenous proteins

interact in melanoma cells and normal dermal human fibroblasts. Over-expression of p16INK4a, BRG1 or both proteins combined revealed that p16INK4a alone caused limited arrest in cells expressing pRb but lacking BRG1. Co-expression of BRG1 with p16INK4a led to complete cell cycle arrest in these cells while BRG1 alone did not affect the cell cycle. Becker's data demonstrate that pRb does not strictly require BRG1 to inhibit proliferation in response to p16INK4a, as was suggested previously. Furthermore, these data show that BRG1 is able to amplify p16INK4a-induced growth arrest through BRG1-INK4a interactions. Becker concluded that BRG1 may be a potentially important tumor suppressor in melanoma, and in fact BRG1 expression is frequently lost in thick primary melanomas.

### Day 3/Session 1. Where are we headed (I):

#### Promising models and pathways. Chair: Lynda Chin

Dr. Chin (Dana Farber Cancer Institute, Boston, MA) opened the session by discussing how integrative genomics such as array-based comparative genome hybridization (array-CGH) has been used to identify novel melanoma oncogenes such as NEDD9. Potential uses for these technologies are the identification of real tumor drivers from genomic bystanders, functional genetic screens for the identification of pre-existing genes in thin versus thick primary melanomas, and genes present in thin melanomas that predispose to metastatic disease.

Glenn Merlino (National Cancer Institute, Bethesda, MD) discussed work that was focused on the earliest initiating stages of melanoma. He presented a new mouse model that can be used to determine the molecular consequences to melanocytes of exposure to UV radiation *in vivo*. In this bitransgenic model, the dopachrome tautomerase (DCT) gene promoter is used to drive melanocyte-specific, doxycycline-inducible expression of GFP. This *in vivo* model is attempting to provide novel insights into the nature of UV-induced "damage," and the mechanisms by which UV provokes melanoma. In addition, using a series of animal- and cell culture-based models of melanoma, Dr. Merlino reported that oncogene-induced senescence, a barrier against early tumor progression, can be overcome by a deficiency in the tumor suppressor ARF, but not p53, facilitating rapid development of melanoma. Accordingly, oncogenic NRAS was found to collaborate with a deficiency in ARF, but not p53, to fully transform melanocytes *in vitro*. This data may help explain in human melanoma the relative abundance and paucity of mutations in ARF and TP53, respectively, and demonstrate that ARF and p53 suppress tumorigenesis through diverse, lineage-dependent mechanisms.

Murray Robinson (Aveo Pharmaceuticals, Cambridge, MA) described the generation of chimeric, embryonic stem cell-based, complex inducible tumor models that can be used for analyzing the variability of human

tumors to drug-induced tumor regression. Specifically, Dr. Robinson detailed how this technology has been used to establish over 100 breast tumors from Her2 overexpressing mice in a null p16INK4a background.

Lewis Cantley (Harvard Medical School, Boston, MA) discussed the role of the phosphoinositide 3-kinase (PI3K) signaling pathway in human cancers. The PI3K enzyme generates PIP3, which in turn controls changes in signaling networks that regulate proliferation and survival. Dr. Cantley also discussed how these networks are modulated by the LKB1 tumor suppressor and upstream activator, or by AMPK, a key sensor of the cell's energy status. LKB1 also negatively regulates mTOR signaling, a pathway activated in a variety of human cancers. Current studies show that increased phospho ERK negatively correlates with phospho AMPK in human melanoma tissues. Future studies should determine whether activators of the AMPK pathway have any benefits for melanoma survival.

### Day 3/Session 2. Where are we headed (II): Evolving therapies. Chair: Frank Haluska

The final session, chaired by Dr. Frank Haluska (Tufts-New England Medical Center, Boston, MA), focused on therapeutic strategies for melanoma, primarily from the drug discovery perspective. Dr. Haluska began by reviewing progress in the discovery of oncogenic mutations in melanoma. He pointed out features of clinical trial design that are important for rapid and decisive evaluation of the exciting pipeline of new agents. Notable clinical caveats were also emphasized, such as the important, yet infrequently highlighted, differences between stage 4 disease involving subcutaneous soft tissues (relatively good prognosis) as compared to visceral metastases (relatively poor prognosis). Recognition of such patient characteristics may be crucial for an ability to draw general conclusions from smaller initial clinical trial results.

Two different kinase development companies presented data on the development of small molecules that target mutated oncogenes. Dr. Joyce James (Ambit Biosciences, San Diego, CA) described a kinome-like library of kinases that are screened against small molecule libraries to rapidly define panels of kinase selectivity. A BRAF inhibitor nanomolar range activity was presented, together with data describing its biological activity relative to other BRAF-targeted agents. Dr. Dan Lynch (Deciphera Pharmaceuticals, Lawrence, KA) provided a review of different classes of kinase inhibitors, based upon differing mechanisms of action (e.g., ATP binding pocket competitive antagonists, allosteric inhibitors, etc.). He described the BRAF<sup>V600E</sup> mutant allele as involving a mutation that is located within a conformational switching pocket, which has been targeted in their drug discovery program. They too have developed a nanomolar BRAF-targeted kinase inhibitor and preclinical data on efficacy were presented.

Dr. Eric Jacobson (Synta Pharmaceuticals, Lexington, MA) presented data on the drug STA-4783, a small molecule that induces oxidative stress. This agent completed Phase II study for stage IV melanoma in combination with paclitaxel and demonstrated a statistically significant increase in progression free survival (3.7 months vs. 1.8 months). The drug is thought to induce production of reactive oxygen species and thereby sensitize cells to apoptotic cell death. Other mechanistic features, including involvement of heat shock response factors were also discussed. Currently a randomized phase III study of this agent in chemotherapy naïve patients is underway. Dr. Dana Aftab (Exelixis, South San Francisco, CA) described a recently developed inhibitor of the KIT kinase. KIT is a receptor tyrosinase kinase that undergoes activating mutations in gastrointestinal stromal tumors and also a small (though significant) subset of melanomas. Two FDA approved small molecule KIT inhibitors have demonstrated significant efficacy against KIT mutated GI stromal tumors, although a set of resistance conferring mutations has been identified. The Exelixis agent, XL820, was presented and suggested to retain activity for multiple mutations within KIT, including several that confer resistance to several other KIT targeted agents.

### **Looking Ahead**

The day after the exciting 4th International Congress of the SMR adjourned, attendees surely awoke realizing

that melanoma remains an inexplicably lethal disease, highly metastatic and predictably resistant to currently available therapeutic modalities. However, if the results of this meeting are any indication, there is much to be positive about and many developments upon which to build for the future. For example, as technological advances in genomics come into sharper focus, a reliable translation is being forged between melanoma genotype and phenotype. The concept of the melanoma stem cell is gaining traction, and may hold the keys to melanoma dormancy, metastasis and drug resistance. Mouse models of various types of melanoma are becoming more sophisticated and more relevant, providing novel mechanistic insights and serving as platforms for preclinical studies. And the promise of BRAF is now perhaps beginning to be realized.

From the clinical perspective, this meeting demonstrated that the technologies required to produce selective and biologically active targeted small molecules are alive and well in the pharmaceutical industry. Moreover, an active pipeline of agents with great pertinence to melanoma currently exists, and is particularly attractive because of mechanism-based correlative studies, which are likely to inform both patient selection and efficacy variables in future clinical studies. The presence of vigorous intermingling among melanoma basic scientists and drug development experts at the 4th International Congress of the SMR produced a buzz of excitement over anticipated progress in the near future.