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Meeting report from the 10th International Congress of the Society for Melanoma Research, Philadelphia, PA, November 2013

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2013 SMR annual meeting report for PCMR

This year’s SMR took place in Philadelphia, PA. This was the tenth anniversary celebration of the Society’s formation and was attended by about 800 registrants, a record-breaking number. The meeting opened with a keynote talk from Joan Brugge (Harvard Medical School, Boston, MA). Dr. Brugge, a noted breast cancer researcher, showed us how her three-dimensional cell culture models can help us better understand how cells in a tissue-like environment respond and resist to therapy. Cells at the outer edges of the breast spheroids or ‘acini’, that is, cells that remained in contact with their extracellular matrix, did not respond to therapy as well as those in the center of the spheroids. RPPA studies showed how tumor cells alter their signaling to overcome the loss of attachment and targeting these pathways could be a potent way of inducing death in tumor cells. The dynamic reprogramming of the kinome in response to MEK inhibition in triple-negative breast cancer was also explored.

Next, David Fisher (Massachusetts General Hospital, Boston, MA) discussed pathways that modulate melanoma formation and survival and showed how UV triggers a sun-seeking behavior through upregulation of MSH and beta-endorphin. An opiate antagonist even caused withdrawal symptoms in chronic UV-irradiated mice. The final focus was on the signaling between the melanocortin receptor, opioid receptor, cAMP, and effects on pain threshold (suggesting that redheads have a higher pain threshold) and how morphine appears to act as an antagonist of the MC4R receptor; these studies may lead to new pain relief strategies.

Targeted therapies in melanoma

The first plenary session was led by Richard Marais (Manchester Institute, Cancer Research, UK) who opened the plenary session by discussing his laboratory's recent work on UV-accelerated BRAF-driven melanomagenesis by targeting a single p53 allele. Using a BRAF-V600E-inducible mouse model, localized BRAF expression was induced by topical treatment with tamoxifen. Four weeks later, half of the back of the mouse was covered and the other half was irradiated with a weekly low dose of UV. Irradiation led to p53 induction in the keratinocytes, thickening of the skin, and clonal expansion of melanocytes only in BRAF-mutant mice. Moreover, UV induced multiple tumors in BRAF-V600E mutant mice in the irradiated area, with 100% penetrance, and decreased latency compared to non-irradiated mice. Interestingly, sunscreen provided some but not complete protection against UV irradiation, as evidenced by fewer tumors and an increase in latency. Whole-exome sequencing of these tumors demonstrated a classical UV signature and gain-of-function p53 mutations. In the second part of his talk, Dr. Marais focused on uveal melanoma. Whole-exome sequencing of these primary melanomas revealed very few mutations, including the known GNAQ and BAP1 mutations. Mutations in SFB3B1, a component of the spliceosome, were also identified in approximately 15% of uveal melanoma samples. RNA sequencing revealed that SF3B1 mutations were associated with alternative splicing of the long non-coding RNA CRNDE and of genes such as ABCC5 and UQCC. Studies are ongoing to understand the biological significance of these findings.

Martin McMahon (Helen Diller Family Comprehensive Cancer Center, UCSF, San Francisco, CA) described a new mouse model of melanoma carrying concurrent PI3K α and BRAFV600E mutations. Mice harboring one mutant Pik3Ca^{H1047R} allele developed melanomas that grew slower than those grown in Tyr::CRE-ER^{T2};Braf^{CA/+}; Pten^{F/F} mice. However, tumors from mice with homozygous Pik3Ca^{H1047R} mutations grew much more rapidly than Braf^{CA/+};Pten^{F/F} tumors, suggesting that strong activation of PI3K can phenocopy the effects of PTEN silencing in murine models. The potential relevance of PIK3Ca mutations or PTEN silencing in conferring sensitivity to PI3K blockade was evaluated using small-molecule inhibitors. Mouse melanoma cells derived from the Braf^{CA}; Pik3Ca^{H1047R} mice were sensitive to BYL719, a PI3K α -selective inhibitor, whereas Braf^{CA};Pten^{F/F} cells were substantially less sensitive to BYL719. Additionally, Braf^{CA}; Pten^{F/F} cells were sensitive to a PI3K β -sparing inhibitor, GDC0032, suggesting that PI3K β may not play a major role in driving PTEN^{-/-} melanomas. Dr. McMahon ended his presentation showing evidence that combined BRAF and PI3K α inhibition caused regression of Braf^{CA}; Pik3Ca^{H1047R} tumors. Similarly, combined inhibition of MEK and PI3K displayed cooperative effects blocking the proliferation of human melanoma cell lines.

A lively discussion by Georgina Long (The University of Sydney, Sydney, Australia) emphasized the success of BRAF inhibitors in melanoma, what lessons have been learned from MAPK pathway inhibition (MAPKi), and how we can use this experience to develop better therapies. She emphasized the need to hit the target early, hard, and persistently. Dr. Long shared data from patient-derived cell lines, suggesting that BRAF inhibition is still useful against BRAF inhibitor (BRAFi)-resistant tumor cells, by slowing their growth compared to resistant cells left untreated. She also emphasized the challenge posed by the

heterogeneity of BRAFi resistance mechanisms and the need to concurrently hit multiple targets including the PI3K pathway, epigenetic changes, and immune processes.

Antoni Ribas (UCLA Medical Center, Los Angeles, CA) explained that tumors treated with immunotherapies or targeted therapies can attract monocytic and granulocytic myeloid-derived suppressor cells (MDSCs) through the secretion of CSF1. Recruitment of these monocytic cells causes an inflammatory microenvironment, supporting tumor growth and an environment that is not permissive to immunotherapy success. Therefore, Ribas hypothesized that inhibition of CSF-1R by PLX3397 could improve the efficacy of current immunotherapies and targeted therapies. Ribas presented data demonstrating that combined treatment with PLX3397 and adoptive T-cell (ATC) transfer resulted in increased tumor growth inhibition compared to PLX3397 or ATC monotherapies. He noted that treatment with PLX3397 decreased macrophage infiltration at the tumor site, allowing for increased functional activity of T cells. Clinical studies based on these data are planned. In the last part of his presentation, Dr. Ribas emphasized the potential of using approaches that can enhance T-cell function and tumor infiltration to improve the benefits of immunotherapies such as anti-PD/PDL1 blockade. He also briefly talked about ongoing studies on combinatorial approaches targeting BRAF and the immune system. He presented data suggesting that a subset of melanoma cells that are intrinsically resistant to BRAFi express high levels of PDL-1. Likewise, melanoma cells that acquire resistance to BRAFi through upregulation of RTKs express higher PDL-1 levels than their isogenic sensitive counterparts. These and other ongoing studies argue that combining BRAFi and immunotherapies could be a potential strategy to overcome drug resistance.

Ahmed Samatar (MERCK Research Laboratories, Boston, MA) discussed the discovery and characterization of SCH772984, the first ERK inhibitor that has moved into early-phase clinical trials. SCH772984 was identified as a selective ERK1/2 inhibitor through a high-throughput screen. Subsequently, co-crystallization showed that this compound also blocked MEK-mediated ERK phosphorylation by distorting the conformation of ERK and masking the MEK phosphorylation sites. SCH-77984 effectively blocked the MAPK pathway and decreased proliferation of a number of BRAF-V600E and KRAS mutant cells in vitro and in vivo. Furthermore, Dr. Samatar presented data demonstrating that SCH772984 overcomes resistance to BRAF and MEK inhibitors and that co-inhibition of BRAF and ERK results in enhanced antitumor activity. A clinical grade analog of SCH77984 is currently undergoing clinical evaluation.

Barbara Bedogni (Case Western Reserve University, Cleveland, OH) presented results indicating that NOTCH directly regulates NRG expression and that the NOTCH-NRG1-ERBB2/3 axis modulates melanoma cell growth. NOTCH and ERBB signaling activate similar signaling pathways including the MAPK and PI3K/AKT pathways. Finally, she presented data indicating that NOTCH and ERBB sustain NF- κ B signaling by inhibiting KEAP1; co-inhibition of NOTCH and ERBB impaired the growth of BRAF wild-type and BRAF-mutant melanomas.

The immunotherapy session was chaired by Drs. Sanjiv Agarwala (Temple University Medical School, Philadelphia, PA) and Patrick Hwu (MD Anderson Cancer Center,

Houston, TX). Five clinical oncologists presented exciting new data obtained in clinical trials in advanced melanoma patients with immune checkpoint inhibitors targeting CTLA-4, PD-1, or PDL-1. Encouragingly, the CTLA-4 inhibitor ipilimumab was recently approved for the treatment of metastatic melanoma patients.

Jedd Wolchok (Memorial Sloan-Kettering Cancer Center, New York, NY) reported a 21% overall survival rate with ipilimumab (anti-CTLA-4 antibody) after 3 yrs of observation. When the antibody was combined with nivolumab (anti-PD-1 antibody), the 1-yr survival rate was 82% and independent of PDL-1 expression by the patients' melanoma cells and absolute lymphocyte counts, but correlated with an increased T-cell activation. In a mouse model, the combination of a BRAF inhibitor and anti-CTLA-4 antibody was more effective in inhibiting tumor growth than either therapy alone. BRAFi enhanced T-cell activation through MAP kinase activation. A randomized phase II study combining ipilimumab with external beam radiotherapy is ongoing, based on the demonstration of decreased myeloid-derived suppressor cell activity after radiotherapy, increased anti-NY-ESO-1 antibody induction, and DR expression by melanoma cells.

Stephen Hodi (Dana-Farber Cancer Institute, Boston, MA) reported that the addition of GM-CSF to ipilimumab significantly increased survival of advanced melanoma patients and decreased toxicity as compared to the antibody alone. Activation of antigen-presenting cells and healing effects on intestinal mucosa by GM-CSF were discussed as possible underlying mechanisms for these effects. In recent trials with a combination of ipilimumab and an angiogenesis inhibitor, tumor infiltrates showed enhanced CCR7+ memory cells, and a 25% stable disease response rate was achieved. A possible role for endothelial cells and their markers in this combination therapy was discussed.

Bob Vonderheide (University of Pennsylvania, Philadelphia, PA) showed a 25% overall response rate in advanced melanoma patients treated with anti-CD40 antibodies combined with anti-CTLA4 antibodies. Activation of antigen-specific T-cell responses was seen in the treated patients. In a mouse model, anti-CTLA-4 therapy was combined with stereotactic body radiation therapy (SBRT) as a means to cause immunogenic cell death. Enhanced tumor regression with increased T-cell infiltrates in tumors was observed in the animals with the combination treatment versus either therapy alone. In a clinical trial in melanoma patients, the combination therapy resulted in an objective response rate of approximately 50%. Clinical trials of a combination of anti-CD40 antibodies, anti-CTLA-4 antibodies, and SBRT are planned.

Caroline Robert (Institut Gustave-Roussy, Villejuif, France) reported tumor reduction in 74% of advanced melanoma patients treated with the humanized anti-PD-1 antibody MK-3475. OS at 12 months of treatment was 81%, ORR was 41%, and treatment-related adverse events were manageable. The median OS has not been reached for any of the three antibody doses evaluated and most responses occurred early. Based on these encouraging results, a phase III trial is planned.

Jeff Sosman (Vanderbilt-Ingram Cancer Center, Nashville, TN), as part of a multicenter trial, has treated 45 metastatic melanoma patients with an engineered anti-PDL-1 antibody in the

absence of dose-limiting toxicities. ORR of 28% (33% in cutaneous melanoma patients) was observed, independent of the intensity of PDL-1 tumor expression, and was highest in patients with prior immunotherapy. Both rapid and delayed responses were seen. Clinically responding patients showed increased tumor infiltrates with gene expression of Th1 type in cases where on-treatment samples were obtained.

Educational session

Keith Flaherty (Massachusetts General Hospital, Boston, MA) presented an overview of the mutations found in melanoma, which were dominated by mutant BRAF. The BRAF inhibitor vemurafenib was shown to remarkably reduce tumor burden in patients with mutant BRAF melanoma. However, resistance mechanisms allow BRAFi bypass via multiple routes, for example CRAF activation, leading to relapse and progression. This hurdle is being addressed by combination therapy, for example BRAF/MEK inhibitor combination, which has been validated for 50% of the melanoma patients. Dr. Flaherty asserted that there is a need to further improve the BRAFi/MEKi combination therapy, utilizing multiple divergent avenues, including optimization of MAPK targeting, targeting of concomitant somatically altered pathways, interception of downstream response pathways, and leveraging the immunologic effects of MAPKi.

Glenn Merlino (National Cancer Institute, Bethesda, MD) addressed the issue of declining success of cancer drugs in phase II clinical trials, and whether the use of improved preclinical animal models can advance this drug discovery scenario. Fresh tumor xenografts (patient-derived xenografts) in NOD/SCID/IL2R⁻-knockout (NSG) mice were 90% effective at correctly predicting clinical efficacy. He showed data regarding limited utility of the subcutaneously transplanted tumor models, as they often fail to predict drug efficacy against invasion and metastasis. He went on to describe the development of a melanoma preclinical genetically engineered mouse (GEM)-derived allograft (GDA) model utilizing stable labeling of tumor cells with GFP and firefly luciferase (ffLuc). These are subsequently transplanted in a mouse model that is pretolerized to GFP/ffLuc by virtue of transgenic expression of GFP and ffLuc in the pituitary gland under the control of the growth hormone promoter (Glowing Head mice). This strategy provides a fully immunocompetent preclinical mouse model that uses imageable markers and allows analysis of immunotherapy as well as targeted therapies.

Patrick Hwu (MD Anderson Cancer Center, Houston, TX) presented data on the success of adoptive cell therapy with antigen-specific T cells. Significant overall response and progression-free survival rates have been achieved by this therapeutic strategy, which seems to be dependent upon the efficiency of T-cell infiltration into the tumor. Administration of BRAFi PLX4720 was shown to increase infiltration of adoptively transferred T cells via inhibition of VEGF production by melanoma cells. Further data showed that shRNA-based knockdown of PTEN induced BRAF-mutant human melanoma cells to resist T-cell killing. Furthermore, PTEN-null tumors exhibited decreased infiltration of T cells. These results suggest that PTEN loss may be a biomarker of immunoresistance. Hwu suggested that combinations of targeted and immune therapies have great potential. In this respect, he

presented data showing that the Hsp90 inhibitor 17AAG synergizes with anti-PDL1 and anti-CTLA4 to restrict tumor growth in a B16 tumor model.

Elizabeth Grimm (MD Anderson Cancer Center, Houston, TX) talked about the role of inflammation in melanoma etiology. She showed that iNOS expression predicts poor prognosis in melanoma patients. In fact, inflammatory marker combination of CD74+ MIF-iNOS predicted a better overall survival. Targeting iNOS is currently being explored as a therapeutic option. She demonstrated the upregulation of IL-1 in human melanoma and showed that IL-1 blockade repressed melanoma cell growth. Consequently, IL-1 and NF- κ B are also being explored as targets. These are part of a wider effort to identify and exploit therapeutic opportunities in pharmacologic attenuation of inflammatory mediators.

Molecular epidemiology

The molecular epidemiology session featured genome-wide studies aiming to address key questions in melanoma risk and tumor development. Tim Bishop (University of Leeds, Leeds, UK) opened the session by presenting extensive efforts including a meta-analysis of genome-wide association studies (GWAS) that consisted of data from the GenoMEL consortium, AMFS, Western Australia, Cambridge, INSERM, and MD Anderson. In total, the study consisted of approximately 10,000 cases with 13,000 controls and enabled the group of researchers to identify three new genetic risk loci (OCA2 on 15q, KLF4 on 9q, and AGR3 on 7p).

Following this, Kevin Brown (NCI, USA) discussed post-GWAS characterization of risk loci, in particular PARP1 on chromosome 1. He provided evidence for SNPs in PARP1 that have a functional effect. Carla Robles-Espinoza (Sanger Institute, Cambridgeshire, UK) then presented data on POT1 as a novel high-penetrance melanoma susceptibility gene. Using whole-exome sequencing of 90 UK and Dutch melanoma pedigrees, segregating POT1 germline mutations were found in four pedigrees. More-over, evidence describing the functional effect of identified mutations was provided.

Yardena Samuels (NCI, USA) then switched focus from germline to somatic genetics. Focusing on whole-exome sequencing data from a comprehensive set of melanoma tumors, she discussed the effect of a novel recurrent synonymous mutation in BCL2L12 that they subsequently showed to affect apoptosis via a miRNA-dependent mechanism. Finally, Julia Newton-Bishop (University of Leeds, Leeds, UK) highlighted the importance of tumor sampling. In the Leeds melanoma cohort which they study, they considered ulceration as a prognostic factor and simultaneously looked at gene expression signatures characteristic of ulcerated melanomas.

In summary, this session highlighted that during the past few years, significant progress has been made in the understanding of the genetic basis of melanoma risk. In addition, identifying the genetic and molecular patterns in tumors contributes to the understanding of the disease.

Drug resistance

Jessie Villanueva (The Wistar Institute, Philadelphia, PA) reported that a newly identified MEK2-Q60P mutation and BRAF amplification were concurrently present in melanoma cell lines resistant to BRAF and MEK inhibitors and in samples obtained from patients who develop resistance to BRAF plus MEK inhibitors. She presented data demonstrating that those two concurrent genetic events both conferred resistance to the combination of BRAF and MEK inhibitors. The resistant tumor cells had sustained MAPK activation and persistent phosphorylation of S6K. S6K is a node of convergence of the MAPK and PI3K pathways, raising the possibility that S6K could be a suitable target for melanoma therapy. Consistently, a triple combination of dabrafenib, trametinib, and the PI3K/mTOR inhibitor GSK2126458, which indirectly targets S6K, led to sustained tumor growth inhibition in vivo. Therefore, combination strategies targeting the MAPK pathway and PI3K/AKT/S6K axis could overcome resistance to BRAF and MEK inhibitors. Villanueva also emphasized the need to further investigate the use of combination approaches targeting these two critical pathways and other tumor vulnerabilities as upfront therapy to prevent or delay the emergence of drug resistance.

Roger Lo (UCLA, Los Angeles, CA) noted that the AKT/PI3K pathway is commonly induced early during treatment with MAPKi, particularly in the context of PTEN loss. This increase in PI3K/AKT correlates with rebound increase in RTKs or the levels of PIP3 on the cell surface. Dr. Lo then gave an overview of mechanisms of acquired resistance, noting that more than 50% of resistant tumors reactivate MAPK, about 4% engage the PTEN/PI3K/AKT pathway, and almost 20% display activation of both pathways. Analysis of resistant tumors identified genetic alternations in the PTEN/PI3K/AKT axis, including a gain-of-function AKT1Q79K mutation, a loss-of-function PIK3R2 N561D mutation, and a novel PTEN M134 deletion, among others. Finally, Dr. Lo focused on the contribution of tumor heterogeneity and genomic diversity, within the same patient or within the same tumor, to acquired drug resistance. This emphasizes the importance of co-targeting multiple pathways at the onset of therapy.

Andrew Aplin (Thomas Jefferson University, Philadelphia, PA) developed a strategy to quantify the activity of the MAPK pathway in real time in vivo. Human melanoma cells transduced with an ERK activity luciferase reporter were injected into athymic mice; these animals were then fed with control chow or chow containing PLX4720 until BRAFi-resistant tumors emerged. Using this model, Dr. Aplin demonstrated heterogeneity in ERK activity in the emerging resistant tumors. Molecular heterogeneity of resistance mechanisms includes de novo BRAF homozygosity, HRASQ61K mutations, and novel BRAF splicing variants.

Clinical updates

This year's SMR meeting offered a rich source of clinically relevant information, with two themes sharing the spot-light: molecularly targeted and immune-mediated therapies. Dramatic therapeutic advances and improved survival ushered by these emerging agents heralded a shift in perception toward melanoma treatments, epitomized in the statement by

Lynn Schuchter: ‘We can start thinking about using the word cure for melanoma’. This is a powerful statement given that prior to 2010 median survival for patients with metastatic melanoma was 6–9 months.

Dr. Schuchter provided an excellent overview of the historic paucity of new drug approvals in melanoma, with DTIC approval in 1975, followed by high-dose interferon in 1995. In contrast, 4 drugs were approved in the last 4 yrs including ipilimumab, vemurafenib, dabrafenib, and trametinib, along with several other promising agents in advanced stages of clinical testing. Although two FDA-approved targeted BRAF inhibitors have significant and comparable efficacy with response rates around 50%, Dr. Schuchter remarked on a distinct side effect profile between the two agents, requiring specific management. As the approaches to BRAF inhibition evolve, so will likely the methods for the detection of activating BRAF mutations. Ryan Sullivan (MGH, Boston, MA) described a blood-based assay for highly sensitive detection of BRAF mutations, with potential utility both in initial detection of BRAF mutations and in monitoring disease activity.

Unlike BRAF inhibition, single-agent-targeted MEK inhibition in cutaneous melanoma has not shown comparable efficacy. However, in uveal melanoma, where BRAF mutations are absent but mutations in GNAQ/11 predominate, single-agent MEK inhibitor selumetinib demonstrated significant responses (50%) and for the first time, markedly improved survival in uveal melanoma. In contrast to single-agent MEK approaches, combination of targeted BRAF/MEK inhibition in BRAF-mutated melanoma continues to show a significant benefit with increased response rates (76%), lower resistance rates, and prolonged survival, while simultaneously displaying less toxicities, particularly cutaneous SCC formation (combination was FDA-approved for BRAF V600E or V600K disease on January 10, 2014). Emerging combinatorial pathway inhibition approaches were well illustrated by a phase I study of vemurafenib and a PI3K inhibition PX-866 in advanced BRAF-mutant solid tumors and melanomas presented by Tara Gangadhar (Hospital of the University of Pennsylvania, Philadelphia, PA). Durable ongoing responses were observed in this study in both treatment-naïve and previously BRAF- or MEK-treated patients, with best responses seen in 62% (8/13) of naïve and 17% (1/13) BAF/MEK-pretreated melanoma patients.

Dr. Schuchter particularly emphasized the emerging data with immune checkpoint targeted therapies, including anti-PD-1 and PD-1L. The rates and durability of clinical responses with immune checkpoint approaches continue to raise the bar for novel melanoma therapeutics. In particular, combination of immune checkpoint inhibitors further enhances the antitumor activity. Recent phase I data where the CTLA-4 inhibitor ipilimumab and PD-1 inhibitor nivolumab were combined demonstrated objective response rates in 53% of patients, all with tumor reduction of 80% or more. Finally, Dr. Schuchter discussed a particularly relevant question: when to select immunotherapy vs. targeted therapy as the first choice of treatment. The emerging consensus would suggest that for highly symptomatic, high burden disease where time may be of essence, targeted therapy may be a better first option, while immunotherapy may be better suited as a first-line therapy for patients with low disease burden and good performance status. With the arrival of additional agents currently in the pipeline, it is highly likely that the treatment of melanoma will continue to rapidly change and improve in both efficacy and tolerability.

Genomics

This session was dominated by the application of high-throughput genomic techniques to understand drug resistance and delivered a sequence of interesting presentations. Levi Garraway (Dana-Farber Institute, Boston, MA) opened the session by discussing treatment resistance. In a recent study on whole-exome sequencing of tumors from 45 patients treated with BRAF inhibitors (vemurafenib or dabrafenib), mutations in genes of the MAPK pathway (e.g., NRAS, MAP2K1 and MAP2K2) were identified to confer resistance to BRAF inhibition. He also highlighted the importance of the melanocyte lineage program in resistance to BRAF inhibitors. Kate Nathanson (Hospital of the University of Pennsylvania, Philadelphia, PA) continued on the same subject investigating genetic alterations in tumors from patients enrolled in clinical trials. Furthermore, she showed data on PTEN as a marker of progression-free survival among patients treated with dabrafenib. Michael Berger (Memorial Sloan-Kettering, New York, NY) talked about the implementation of next-generation sequencing in a diagnostic setting. In clinical samples, they are investigating somatic mutations in 340 cancer genes. In a subset of patients, genomic results from multiple metastases were available highlighting the complexity of genetic heterogeneity in melanoma. Jeff Trent (TGen, Phoenix, AZ) then described their comprehensive Stand Up 2 Cancer multidisciplinary study that focuses on BRAF- and NRAS-negative melanomas with the aim of comparing tumor-based personalized treatment to standard care of patients with such tumors.

Maria Teresa Landi (NCI, USA) then switched to germline genomics and highlighted for the second time during this meeting POT1 as a high-penetrance melanoma susceptibility gene. In a set of 56 Italian melanoma pedigrees, a single missense mutation was found in five families suggesting it being a founder mutation in this population. Finally, Malin Pedersen from Richard Marais's laboratory (The Paterson Institute for Cancer Research, Manchester, UK) discussed her findings on the expression of NRAS^{G12D} in melanocytes during development. Interestingly, her mouse models developed brain melanomas and a subsequent follow-up in a primary CNS melanoma demonstrated a NRAS^{Q61} mutation, suggesting the involvement of NRAS in the development of brain melanomas.

In conclusion, the genomics session solidified the importance of genomic information from tumors, also with respect to drug resistance, and suggests the imminent introduction of high-throughput techniques in the clinical setting.

Public and private partnerships

Christopher Austin (NIH, USA) talked about the National Center for the Advancement of Technology and Science (NCATS). NCATS is designed to bring together government, academia, industry, philanthropy, and patient advocacy to create an efficient way to overcome current roadblocks in therapy. He discussed the importance of capitalizing on the strengths of each group in order to reengineer and improve the translational medicine process.

Perry Nisen (GlaxoSmithKline, Brentford, UK) also suggested that during this time of budget cuts and tight funding, the marriage of academia and industry is critical and needs to be rewired to be the most efficacious and build upon the strengths of each domain. He spoke about open innovation, data sharing, flexible partnerships, and risk sharing. James Doroshow (Center for Cancer Research, NCI) discussed the need for a better understanding of therapeutic targets and effects in order to develop improved clinical trials. To further efforts in this area, his group has combined FDA-approved drugs for screening cell lines from the NCI cell bank. Because these cell lines have been extensively analyzed, their response can be assessed according to known mutations, molecular changes, and previous data analyses. Using this approach, Doroshow and colleagues have already identified promising drug combinations.

Women in science session

This year we initiated the first of what we hope will be many sessions aimed toward to boosting the participation of women in melanoma research. The goal is to make everyone aware of the unique issues that face women scientists. Interestingly, as times change, some of the challenges that women face today are rapidly becoming generalizable to anyone, man or woman, specifically in the area of work/life balance. Understanding and addressing these issues are critical as we move forward.

Dr. Margaret Foti (American Association for Cancer Research) gave an inspiring keynote speech, highlighting important women scientists in cancer research. She showed data that indicate that while we have come a long way, there is still some work required to close the gaps and achieve gender equality in the scientific arena. Dr. Foti listed some of the root causes of gender inequality in the workplace. These included subjectivity in hiring and performance evaluation, fewer institutional resources, resulting in fewer publications, and ultimately less funding. Additionally, fewer networking opportunities are available to women, and women are less likely to be recognized than men for their accomplishments. She mentioned that the AACR has long been involved in these efforts, having founded the Women In Cancer Research council over three decades ago.

Dr. Foti made the interesting point that women need to help women, and it is one that deserves serious consideration. She quoted Madeline Albright who stated 'There is a special place in hell for women who don't help other women.' Many young women scientists feel that their senior colleagues, both men and women, do not mentor them or try to open up professional opportunities for them. Ideally, both women and men should aspire to be great mentors as exhibited by the qualities Dr. Foti listed: accessible, empathetic, open-minded, consistent, patient, sincere, honest, savvy. Simply put, she said, to echo the words of Prime Minister Shinzo Abe, integration of women into the workforce is just good business. Therefore, it is critical to create guidelines that celebrate the accomplishments of women, and help them to network and succeed. None of this can happen without a strong commitment from those in charge. Scientific societies, such as the SMR, she stated, bear significant responsibility in removing the bias and barriers that exist for women in science today. This can be achieved through more rigorous inclusion of women in scientific

programs, and consideration of women candidates for scientific awards, and leadership positions within the societies.

Dr. Stephanie Abbuhl (University of Pennsylvania) then gave an overview on the issues facing women in academic medicine. She referred to the problem as 1000 pounds of feathers multiple little issues that lead to a significant weight. Some examples include: unconscious bias, cost of assertiveness, mentorship, salary discrepancies, balancing work and home, institutional practices that are out of step with modern life. Particularly intriguing was her breakdown on how women who are ambitious, or assertive are perceived as less feminine, and therefore, the quality of assertion is less desirable in women than in men. Perhaps because of this, women are more prone than men to undergo ‘imposter syndrome’ where they feel like they do not deserve the success they earn.

Work/life balance continues to be a challenge for women, and while it is becoming one for men as well, Dr. Abbuhl cited data suggest that women who live in a double-income household end up doing significantly more household chores. A striking statistic that she cited was that only 5% of female assistant professors have a stay at home spouse, where 49% of male assistant professors have a stay at home spouse. Both Dr. Foti and Dr. Abbuhl encouraged the women in the audience to demand more help from their husbands if they felt it necessary.

What are the solutions to this? Dr. Abbuhl suggested we need to work both from the bottom up, building skills, increasing communications, exposing young scientists to good role models. In addition, we need to work from the top down, with leadership-driven commitment, incentives, and policies.

These enlightening talks were followed by a lively panel discussion, led by Dr. Maureen Murphy. She encouraged women to step up as mentors, as it is empowering, and also to be proactive. Dr. Weeraratna echoed this, encouraging young women to not get weighed down by all the statistics they were faced with in this session, but instead to take personal responsibility for their own success, and not be afraid to ask for what they felt they needed whether from spouses, bosses, or employees. Dr. Schuchter ended the session by providing the charge to ‘move outside your comfort zone, you are incredibly talented’. We look forward to hearing our European colleagues’ take on this situation at the 2014 meeting.

Immunotherapy II

Talks during this session were focused on overcoming therapy resistance. Discussions highlighted immunoregulators of the tumor microenvironment (TME) and strategies to overcome TME-induced resistance mechanisms. Chiara Castelli (Istituto Nazionale dei Tumori, Milan, Italy) described multilevel mechanisms of immune suppression in melanoma patients with emphasis on immune regulatory cells such as plasmacytoid dendritic cells and myeloid-derived suppressor cells (MDSCs) and their induction in the TME. A role for exosomes and other tumor-derived factors in the induction of MDSCs was discussed.

Laszlo Radvanyi (MD Anderson Cancer Center, Houston, TX) presented data on predictive biomarkers for adoptive TIL therapy of Stage III–IV metastatic melanoma patients. Over 75

metastatic melanoma patients were treated with autologous TIL therapy with an approximately 50% response rate and >36 months' median survival; however, not all patients' tumors yielded sufficient numbers of TIL for expansion. A number of biomarkers associated with sufficient TIL outgrowth were identified; these include markers in the original tumors, such as CD3, CD8, and CD4 defined by immunohistochemistry, and expression of immune-related genes such as genes encoding the type I IFN, and unexpectedly, genes associated with immune suppression such as Foxp3. Other genes, such as IRAK1, associated with the activation of TLR and ERK and closely linked with other inflammatory-related factors such as iNOS, IL-1, TNF, IL-6, and miR146a, were expressed at higher levels in tumors from patients failing to respond to TIL therapy. The possible use of drugs targeting inflammatory pathways was discussed to improve therapeutic efficiency of adoptive TIL therapy.

Tom Gajewski (The University of Chicago Medicine, Chicago, IL) stated that one-third of melanoma patients have subsets of T cells expressing immune regulatory molecules such as PD-1, 4-1BB, and LAG3. These tumors also contain FOXP3+ Tregs and IDO. Presence of these molecules or factors rendered T cells dysfunctional. Use of anti-PD-1 and PD-L1 antibodies to overcome immune resistance mechanisms, and the rationale for combination therapies was also discussed.

Rajasekharan Somasundaram (The Wistar Institute, Philadelphia, PA) discussed the role of tumor-associated B (TAB) cells with an inflammatory cytokine phenotype in tumor heterogeneity and drug resistance of melanoma cells. He presented a novel mechanism of acquired drug resistance in melanomas induced by TAB cells involving FGF-2/FGFR-3 and IGF-1 signaling. TAB-cell derived IGF-1 induces therapy-resistant heterogeneous melanoma subpopulations, and neutralization of IGF-1 reverses this induction. Further, knockdown of FGFR-3 in tumor cells restores the sensitivity of melanoma cells to chemo- and targeted therapies.

Zachary Cooper (Massachusetts General Hospital, Boston, MA) described the role of immune checkpoint blockade in enhancing BRAF inhibitor therapy response in a murine model. He briefly summarized the critical role of immune response after BRAF inhibitor therapy. Following BRAF inhibitor therapy, enhancement of granzyme B-positive CD8+ T-cell infiltration and expression of melanoma-associated antigens on tumor cells were observed. The addition of PD-1 or PD-L1 blockade to BRAF inhibitors enhances therapeutic response in this mouse model and was associated with an increase in infiltrate and function of CD8+ T cells.

Biomarkers

Boris Bastian (University of California, San Francisco, CA) addressed the question of whether metastatic spread cascades from regional to distant sites. Whole-exome sequencing comparisons of primary tumor, locoregional metastases, and parotid metastases revealed that the evolution of primary tumors and metastases separates early and that regional and distant metastases evolve separately. He asserted that metastatic dissemination occurs early during progression and can seed distant metastases without exhibiting locally confined intermediate

stages. This observation suggests that in high-risk cancers, there may be a need to deploy systemic therapy at early stages.

Daniel Peeper (Netherlands Cancer Institute, Amsterdam, Netherlands) is researching the role of oncogene-induced senescence (OIS) in melanoma, its mechanisms, and how it can be exploited as a therapeutic strategy. Senescence is a bona fide melanoma suppressor mechanism, which can be overcome by the loss of PTEN. He showed that inhibition of PI3K can restore senescence and synergizes with BRAFi. He went on to show that pyruvate dehydrogenase (PDH) plays an important role in mutant BRAF-induced senescence. Upregulated PDH kinase 1 (PDK1) inhibits PDH and results in OIS bypass. PDK1 knockdown inhibited melanoma tumor growth in a transplant model. These results suggest that PDK1 may be a viable target for combination therapy of melanoma. He also described a parallel in vivo and in vitro shRNA dropout screen for genes required for melanoma expansion. shRNA targeting the DNA damage response (DDR) gene CHEK1 was shown to inhibit tumor growth. It was also shown that hypoxia and HIF1- α stabilization sensitize to DDR inhibition.

Rhoda Alani (Boston University, Boston, MA) talked about exploring molecular pathways associated with tumor progression to leverage data for translational aims. She stressed the critical need for biomarkers in melanoma to improve diagnostic accuracy, determine prognosis, and monitor tumor recurrence. She described a screen for melanoma–endothelial cell crosstalk signatures, in which neuropilin-2 was identified as a high priority biomarker. NRP2 is highly expressed in metastatic melanoma, as well as in melanoma versus other cancers. Quantitative RT-PCR analyses showed high expression of NRP2 in melanocytic lesions, but not in benign nevi. It was concluded that NRP2 may serve as a supplemental tool to distinguish malignant lesions in equivocal diagnostic cases. Further data showed that HELLIS is upregulated, while HGF inhibitor (HGF α) is downregulated in metastatic melanoma. Blood biomarkers of melanoma are also being explored, including circulating cell-free nucleic acids and circulating melanoma cells, as well as serum levels of NRP2. To accomplish biomolecular detection of these biomarkers, interferometric reflectance imaging sensor assays are being developed.

Eva Hernando (New York University, New York, NY) described a microRNA-based signature as a predictive biomarker of melanoma brain metastasis. Multivariate regression logistic analysis, including tumor stage as a continuous variable, yielded a signature of five miRNAs that could effectively distinguish cases that preferably metastasize to brain. Among these 5 miRNAs, miR-150 was shown to have a direct correlation with number of tumor-infiltrating lymphocytes and inversely with the development of brain metastasis.

Christopher Moy (GlaxoSmithKline, Collegeville, PA) described a study focused on BRAF amplification upon response to the MEK inhibitor trametinib. Next-generation sequencing and copy number profiling were performed on archival biopsies of 47 patient samples. A significant relationship was found between BRAF gain and limited response in patients with BRAF-V600E mutation.

Iman Osman (New York University Langone Medical Center, New York, NY), discussed melanoma brain metastases and the pros and cons of several preclinical in vivo mouse models of brain metastases. The potential role of JAK-STAT, MMPs, bFGF, VEGF, endothelin receptor B, anti-apoptotic protein (BCL2A1), Connexin 26, CCR4, and TGF in contributing to brain metastasis was also discussed. Suzanne Topalian (John Hopkins University, Baltimore, MD) gave an update on the clinical activity of PD-1 (nivolumab and MK-3475) and PD-L1 (BMS-936559 and MPDL3280A) blocking antibodies in melanoma and other cancers. PD-L1 expression in pretreatment tumor biopsies correlated with good clinical response to anti-PD-1 therapy. Responses to anti-PD-1 are associated with CD8+ T-cell infiltration. As shown by others, BRAF inhibitors enhance infiltration of CD8+ T cells and upregulate tumor PD-L1 expression; thus, BRAF inhibitors can be effectively used in combination with anti-PD-1 therapy for an additive effect.

Marcus Bosenberg (Yale School of Medicine, New Haven, CT) presented high-throughput screening techniques to analyze combinations of small-molecule inhibitors. Promising drug combinations targeting receptor tyrosine kinase, BRAF, or RAS mutations were discovered in the study. Mateo Carlino (Westmead Millennium Institute, Sydney, Australia) showed that ERK inhibitors are more active than a MEK inhibitor (trametinib) as they are more effective in inducing cell cycle arrest. Negative feedback loop activation of the PI3K/AKT pathway impedes ERK inhibitor-induced apoptosis, and also ERK inhibitors are more effective in combinations of dual pathway blockade. Using a genetically engineered mouse model of BRAF and in vivo insertional mutagenesis, Daniele Perena (Beth Israel Deaconess Cancer Center, Boston, MA) showed that both *Braf* and *ERas* are top candidate genes playing a causal role in resistance to PLX4720. Further, *ERas*-mediated resistance is through activation of the PI3K/AKT/BAD pathway and can be reversed by combination therapy with AKT/PI3K inhibitors. Katrina Meeth (Yale School of Medicine, New Haven, CT) discussed the in vivo inhibition of macrophages using PLX3397 or GW2580 (both specific inhibitors of CSF1R on macrophages) in combination with BRAF inhibitors, which delayed tumor growth. Altering macrophage recruitment by blocking CCR2-CCL2 signaling had the same outcome.

Pigmentation/UV/DNA repair

Bill Pavan (National Human Genome Research Institute, NIH) discussed genomic approaches from his laboratory to understand the chromatin landscape in melanocytes and how it is influenced by genetic and environmental factors. Using ChIP-seq-based approaches, his group and collaborators have identified a set of enhancer elements that they have used to predict sequence motifs common to the melanocyte lineage. In addition, they were able to predict when variations have deleterious effects on gene expression, giving IRF4 as an example. Dr. Pavan also discussed future studies to integrate additional transcription factors and chromatin biomarkers as a useful resource for the melanoma community.

Lionel Larue (Institut Curie, Paris, France) spoke of an alternative pathway inducing the nuclearization of β -catenin, which is independent of GSK3. He showed the lack of PTEN-induced β -catenin transactivation and subsequent repression of senescence-inducing

p16INK4A expression. Surprisingly, increased PI3K signaling toward GSK3 activation only played a minor role in this process. Rather, loss of PTEN increased Caveolin-1-mediated dissociation of membranous β -catenin driving its nuclear translocation and inhibition of p16INK4A-induced senescence. These findings indicate that during multistep melanomagenesis, loss of PTEN cooperates with MAPK signaling by enabling a novel, Caveolin-1-mediated, mechanism of senescence suppression.

Alain Mauviel (Institut Curie, France) spoke of GLI2, a Kruppel-like transcription factor usually associated with Hedgehog (HH) signaling, as a direct TGF- β /SMAD target in cutaneous melanoma progression. High GLI2 expression was independent of HH signaling in the most invasive melanoma, depending instead upon autocrine TGF- β signaling; this was associated with metastatic features. Mauviel's work demonstrated that GLI expression in tumors should not be solely regarded as a marker of HH pathway activation and instead may occur because of exacerbated TGF- β signaling.

Corine Bertolotto (INSERM, France) demonstrated that germline mutations in MITF (MITF 318K) leads to disrupted SUMOylation and predisposition to melanoma. MITF suppression mediated senescence-like phenotypes in melanoma cells, and Bertolotto provided evidence that MITF E318K, which predisposes carriers to melanoma, exerts its pro-tumoral effect by preventing a senescence program.

Sandra Pavey (University of Queensland Diamantina Institute, Brisbane, Australia) spoke about the G2 phase cell cycle checkpoint and repair in response to suberythemal UV exposure. Pavey and colleagues investigated the global alterations at the transcriptional (total mRNA) and translation level (polysome-bound mRNA) associated with the UV G2 phase checkpoint, to identify the normal components of the checkpoint and repair pathways involved in an A2058 melanoma cell line model and a whole skin organ culture model.

Juxiang Cao (Boston University, Boston, MA) presented evidence that DOT1L, a mammalian homolog of yeast DOT1 (disruptor of telomeric silencing), assists UV-induced DNA damage repair in melanocytes. Under UV irradiation, the DOT1L-mediated H3K79 dimethylation (H3K79-2Me) accumulated at DNA damage sites and recruited NER factors to the DNA damage sites in melanocytes. Dr. Cao demonstrated that DOT1L deficiency did not affect the expression of NER factors, but did impair the recruitment of NER factors to sites of DNA damage, indicating that DOT1L has a novel function in NER after UV irradiation.

Zalfa Abdel-Malek (University of Cincinnati Cancer Institute, Cincinnati, OH) discussed that in addition to alpha-MSH, the paracrine factors endothelin-1 and 1,25(OH)₂ vitamin D3 (both of which are synthesized by keratinocytes) and neuregulin-1 (NG1; synthesized mainly by fibroblasts) modulate the response of epidermal melanocytes to UV radiation. These three paracrine factors have similar effects on the UV-induced DNA damage in melanocytes by diminishing the generation of reactive oxygen species, enhancing repair of DNA photo-products and oxidative DNA damage, and inhibiting apoptosis. Dr. Abdel-Malek suggested that these paracrine factors help restore genomic stability of melanocytes upon UV exposure.

Tumor microenvironment and metastasis

The tumor microenvironment is currently an area of immense interest. Ze'ev Ronai (Sanford-Burnham Medical Research Institute at La Jolla, San Diego, CA) began the session by discussing the importance of establishing the role of PDK1 as a new target for the development of metastasis. Inactivation of PDK1 in the BRAF/PTEN/cdkn2a mouse delays melanomagenesis and prolongs survival of the mutant animals. Further, the top PDK1-dependent pathways altered in BRAF/PTEN melanomas reveal a FOXO signature with PDK1 mutant melanomas exhibiting decreased AKT phosphorylation and increased nuclear FOXO3. FOXO3A has been shown to be an important player in BRAF/PTEN melanomas and inhibition of FOXO3A in PDK KO/BRAFV600E/PTEN-knockout melanomas partially rescues proliferation and colony formation. Finally, the use of PDK1 inhibitors (such as GSK-2334470) in treating melanoma was discussed. Inhibition of PDK1 attenuates and inhibits metastasis, and this was proposed as a combination therapy with BRAF inhibitors.

Gavin Robertson (Penn State College of Medicine, Hershey, PA) focused on the role of circulating tumor cells and their interactions with the microenvironment. Specifically, IL-8, expressed by circulating melanoma cells, recruits neutrophils by acting as an anchor. In an animal model, it was demonstrated that neutrophils help retain circulating melanoma cells in the lungs and increased IL-8 was present in metastatic circulating tumor cells. IL-8-knockout studies demonstrated a decrease in the aforementioned affects, demonstrating that IL-8 can modulate circulating melanoma cell metastasis. Dr. Robertson went on to discuss the role of the TGF- β family member, macrophage inhibitor cytokine-1 (MIC-1), which is elevated in melanoma patients and melanoma cell lines. Decreased MIC1 secretion (siRNA) reduces the invasiveness and metastasis of melanoma cells and the addition of recombinant MIC1 increased invasiveness, thus also modulating melanoma circulating tumor cell metastasis.

An area of increasing interest is the role of the aging microenvironment in contributing to disease pathogenesis. This was discussed by Ashani Weeraratna (The Wistar Institute, Philadelphia, PA). An aging microenvironment was demonstrated to be pro-invasive and prometastatic to melanoma cells achieved, in part, by factors secreted by the surrounding aged fibroblasts. Further discussion surrounded highly invasive melanoma cells that respond differently to DNA damage, a factor involved in the aging microenvironment. Highly invasive cells have intact cell cycle markers and p21 appears to be relatively functional and can be activated by irradiation. Interestingly, Wnt5A high cells appear to be senescent, but Wnt5A can continue to drive invasion. These senescent-like melanoma cells can form colonies in vitro and in vivo and the metastatic lung colonies retain senescence-associated beta-galactosidase (beta-gal) positivity. Importantly, after BRAF inhibitor treatment, the remaining cells are beta-gal positive, implying that a senescent-like arrest may be an adaptive stress response to multiple forms of therapy.

The session continued with Sheri Holmen (Surgery, University of Utah, Salt Lake City, UT) discussing how differentially activated AKT isoforms in a mouse model of melanoma influence metastatic potential. All AKT isoforms induced melanoma in the mouse model but there was a significant difference between the isoforms and the metastatic phenotypes. The

AKT2 isoform mice had a shorter lifespan because the tumors grew quickly, while the AKT3 mice had to be sacrificed due to brain metastases, but the primary tumors were relatively small. These studies highlight the importance of evaluating the effect of targeting different AKTs, especially with regard to metastatic potential.

This was followed by Daniel Zingg (Institute of Anatomy, University of Zurich, Zurich, Switzerland) who presented data on the epigenetic modifier EZH2, a methyl transferase that triple methylates lysine 27 of histone H3, and its requirement for melanoma metastasis. EZH2 is overexpressed in many cancers and provides the capacity for tumor cells to shut down certain genes. EZH2 expression is increased in human melanoma and is associated with poor survival. These studies compared nevi to human subcutaneous melanoma metastases using SOX10 as a marker of cells of the melanocytic lineage. In order to look at EZH2 function, a transgenic melanoma mouse model was used. EZH2 expression is increased in melanomas from the Tyr:NRasQ61K Ink4a $-/-$ mouse model. Targeted EZH2 deletion, induced with tamoxifen, led to stabilization or regression of the disease and halted invasive behavior. Finally, treatment of established melanoma with the EZH2 inhibitor GSK503 led to stabilization of the disease.

The session was concluded by Maria Garcia-Fernandez (CNIO, Madrid, Spain) demonstrating selective roles of autophagy in melanoma metastasis, defined by genetically engineered conditional mouse models. The generation of inducible ATG5-deficient mouse melanoma models was discussed. ATG5 is a central mediator in autophagy programs, and ATG5 deletion did not affect nevi formation; however, there was a delayed generation of cutaneous lesions in ATG5-null mice. Ultimately, all mice developed cutaneous melanoma independently of ATG5 background and there were no differences between males and females. In sum, reduced ATG5 copy number in human melanoma cells shows no significant contribution to benign hyperplasia, while heterozygous deletion actually enhances metastatic development.

Tumor heterogeneity and plasticity

The final session of the meeting was on another area of increasingly popular interest: tumor heterogeneity and plasticity. Mizuho Fukunaga-Kalabis (The Wistar Institute, Philadelphia, PA) discussed how canonical Wnt signals derived from keratinocytes could inhibit the Notch pathway to specify human neural crest-like stem cell fate. Notch 1 or MSX1 can reprogram mature melanocytes to neural crest-like multipotent stem cells and inhibition of Notch signaling reduced proliferation of neural crest-like spheres and induced cell death. It was shown that Wnt1 and Wnt3a promote the development of neural crest cells into pigment cells and that Wnt3a activates the expression of MITF. Wnt3a is required in human embryonic stem cells for melanocyte differentiation and Wnt signaling regulates that differentiation. Interestingly, Wnt7a promoted melanocyte differentiation from neural crest-like stem cells and activates both the canonical and the non-canonical pathways in a context-dependent manner. Finally, keratinocyte-derived Wnt signals were shown to antagonize Notch activity in neural crest-like stem cells, which can be blocked by two different inhibitors (XAV939 and NSC668036).

Next, Claudia Wellbrock (University of Manchester, Manchester, UK) discussed how MITF is overexpressed in a majority of resistant cells and demonstrated that MITF overexpression can actually lead to resistance. In addition, reduction of MITF increases sensitivity to MEK inhibitors. In the majority of patients during treatment with BRAFi, MITF expression was upregulated in response to treatment. Based on this, targeting MITF regulation may be a beneficial therapeutic approach. Further, it was demonstrated that TNF- α upregulates MITF and protects cells from MEK inhibitor-induced apoptosis.

Michael O'Connell (The Wistar Institute, Philadelphia, PA) then discussed the role of non-canonical Wnt signaling in driving phenotypic plasticity in melanoma cells, which can be further regulated by hypoxic conditions. Melanoma cells switch from an ROR1 receptor positivity to an ROR2 receptor positivity as the disease progresses and a hypoxic microenvironment can exacerbate the switch. Importantly, this switch was also seen in therapy resistance. Patients that demonstrated high Wnt5A/ROR2 showed less clinical response to BRAF inhibitors and Wnt5A/ROR2 levels were increased in melanomas that had acquired BRAFi resistance. Further, shutting down Wnt5A signaling by knocking out its receptor, ROR2, increased melanoma cells' response to BRAF inhibitor treatment in vitro and in vivo. This suggests that targeting the Wnt5A pathway in combination with the MAPK pathway increases tumor cell sensitivity to currently available therapies.

Next, Colin Goding (University of Oxford, Oxford, UK) talked about the role of MITF in regulating melanoma heterogeneity and asked whether better therapies can be developed by taking heterogeneity into account. MAPK-driven acetylation of MITF leads to distinctions between melanocyte differentiation and a more proliferative phenotype. MITF was acetylated following MAPKi, leading to the hypothesis that preventing acetylation could possibly increase sensitivity to currently available inhibitors. In addition, it was demonstrated that in response to MAPK signaling, MITF comes off low-affinity binding sites to attach to higher-affinity binding sites. This affinity differs in vivo between the different classes of MITF targets. When MITF is a high-affinity binder (non-acetylated), one gene set is upregulated; meanwhile, acetylated MITF becomes a lower-affinity binder and affects a different gene set.

Next, Alexander Roesch (The Saarland University Hospital, Homburg/Saar, Germany) talked about over-coming intrinsic multidrug resistance through blockage of the mitochondrial respiratory chain of JARID1Bhigh cells. JARID1B, a marker for a slow cycling cell subpopulation in melanoma, acts as a binding partner for Rb and is heterogeneously expressed in melanoma samples and cell lines. JARID1B is required for continuous melanoma growth and expression is determined by the microenvironment, as hypoxia leads to its upregulation. JARID1B-positive cells are intrinsically resistant to chemotherapeutics and vemurafenib; additionally, drug treatment leads to upregulation of JARID1B-positive cells in surviving cell populations. Finally, the slow cycling phenotype is dependent on mitochondrial bioenergy supply, as there is an upregulation of mitochondrial proteins involved in the electron transport chain. Targeting JARID1B-positive cells by phenformin in vivo leads to an improvement in therapeutic response; thus, metabolism is important for therapeutic resistance. In summary, there is an advantage to therapeutic strategies that deplete critical, intrinsically resistant, tumor cell subpopulations.

Robert Ballotti (INSERM, Nice, France) presented data suggesting that in order to treat melanoma effectively, we need to push cells toward a 'monopopulation' to increase treatment sensitivity. Dr. Ballotti showed that loss of MITF, which can be induced by hypoxia, is sufficient to increase the tumorigenic potential of melanoma cells; meanwhile, the MITF-negative cell population is required for melanoma tumor formation. Further, he showed that senescent melanoma cells produce a protumoral secretome demonstrating an increase in a mesenchymal phenotype. The pro-tumorigenic effects of the secretome from senescent melanoma cells require STAT3 activation.

The final presentation of the meeting was by Meenhard Herlyn (The Wistar Institute, Philadelphia, PA) who discussed microenvironmental and treatment signals that can stimulate tumor heterogeneity and therapy resistance. Data presented focused on signaling inhibitors that can induce senescence in some melanoma cells but this senescence can be reversible (transient) or irreversible; this response involves autophagy. The senescent phenotype was also shown to be induced by BRAF and combination BRAF/MEK inhibitors. Senescent cells displayed oxidative phosphorylation pathway signaling, and using a mitochondrial HSP90 inhibitor (gamitrinib) in combination with a BRAF inhibitor improved therapeutic response. Indeed, combination of PLX4720 and gamitrinib enhanced tumor growth inhibition in vivo. This work suggested that in order to treat melanoma successfully, subpopulation of cells that can survive currently available therapies must be targeted.

Late breaking abstracts

Robert Andtbacka (Huntsman Cancer Institute, Salt Lake City, UT) reported the results of a phase III late-stage melanoma (n = 390) plasmid DNA-based immunotherapy for metastatic melanoma. In this trial, the Velimogene aliplasmid was injected intralesionally and compared to DTIC or TMZ. While DTIC/TMZ was more effective than the Velimogene aliplasmid, having the best overall response rate at >24 weeks in this trial, the responses with the Velimogene aliplasmid were more durable. Dr. Andtbacka pointed out that the treatment landscape for metastatic melanoma is rapidly changing and that recently approved or investigational follow-on treatments may have affected overall survival. Thus, chemotherapy may not be an appropriate control in an immunotherapeutic treatment study.

Adil Daud (UCSF, San Francisco, CA) provided an update on a randomized phase II trial in BRAF V600E/K metastasized melanoma (n = 162) comparing monotherapy dabrafenib (D) 150 mg BID, D+ trametinib (T) 150/1QD, and D+T 150/2QD in 3 arms. The combined D+T safety profile was tolerable and manageable, with most common AEs being pyrexia, fatigue, and chills; the pyrexia could be managed with dose interruption or reduction, with the use of antipyretics, and in some cases corticosteroids. The updated median OS was 23.8 months for the 150/2 combination and the OS trend in favor of D+T (150/2) vs mono D although 83% of subjects on mono D crossed over. Interestingly, treatment with the combination beyond progressive disease appears to show a benefit in overall survival. Phase III studies are ongoing.

Howard Kaufman (Rush University Medical Center, Chicago, IL) provided an interim overall survival subset analysis of a phase III trial of intralesional talimogene laherparepvec

(T-VEC, an oncolytic immunotherapy) compared to subcutaneously administered GM-CSF. T-VEC showed a significant improvement over GM-CSF for the primary endpoint of durable response rate, defined as a response lasting at least 6 months continuously (GM-CSF DRR 2.1% n = 141, T-VEC DRR 16.3% n = 295). At last assessment, the majority of responders had not yet developed resistance. The effect was pronounced in patients with stage IIIB-IVM1a disease and in patients receiving T-VEC as first-line therapy. Work to further investigate T-VEC as an immunotherapy is currently underway.

Conclusion

Overall, the meeting showcased the novel molecular findings involved in drug resistance, the advances in immunotherapy, and the possibility of combining these approaches. We also learned about the critical role of genetics and heterogeneity in driving tumor progression and response to therapies. We look forward to hearing about the strides made in these areas and others at the next meeting in Zurich.