Highlights of Novel Melanoma Therapies
presented at the
8th International Congress
of the
Society for Melanoma Research (SMR)
Tampa, FL, November 9-11, 2011

Dorothee Herlyn, DVM, DSc, The Wistar Institute, Philadelphia, PA 19096

After decades of melanoma therapy with the FDA-approved immunomodulators interferon-alpha and interleukin-2, and the chemotherapeutic agent, dacarbazine (DTIC), two new compounds we approved in 2011. One targets mutated BRAF kinase, which is expressed by ~50% of melanomas and not normal tissues. The other targets CTLA-4, an inhibitory molecule on activated T cells. The 2011 SMR meeting focused, among other important aspects of melanoma, on these two treatment modalities which are reviewed below.

Targeting mutated BRAF

Vemurafenib (Zelboraf) is a small molecule kinase inhibitor that specifically targets mutated BRAF (V600E mutation). It is indicated for the treatment of patients with unresectable metastatic melanoma harboring this mutation. Enhancement of progression-free and overall survival of metastatic melanoma patients after treatment varied in different clinical trials (K. Flaherty, Massachusetts General Hospital, Boston, MA). Vemurafenib treatment significantly reduced risk of mortality and tumor progression, compared with standard DTIC chemotherapy (P. Chapman, Sloan Kettering Cancer Center, New York, NY). Very little is known about possible mechanisms of tumor growth inhibition by BRAF-V600E inhibitors, other than direct anti-proliferative effects. Up regulation of melanoma-associated antigens, followed by increased T cell infiltration of tumors, is one possibility (D. Fisher, Massachusetts General Hospital, Boston, MA).

Most patients relapsed after treatment, however, prompting investigations on the mechanism(s) of BRAF-V600E resistance and on new combination treatments to overcome this resistance. Resistance may not be due to V600E mutation loss after targeting this molecule (M. Davies, UT MD Anderson Cancer Center, Houston, TX). Primary events leading to BRAF-V600E inhibitor resistance are due to mutation of NRAS (R. Lo, UCLA, Los Angeles, CA), leading to enhanced signaling through CRAF. CRAF activation leads to activation of other kinases, such as ERK (L. Garraway, Dana Farber Cancer Institute, Boston, MA; J. Villanueva, The Wistar Institute, Philadelphia, PA), MEK (K. Flaherty, Massachusetts General Hospital, Boston, MA), IGF1R/PI3 kinase (J. Villanueva, The Wistar Institute, Philadelphia, PA) or CRAF (R. Lo, UCLA, Los Angeles, CA; J. Villanueva, The Wistar Institute, Philadelphia, PA).
Based on these observations, clinical trials with combinations of BRAF-V600E inhibitors on the one hand and ERK, MEK, PI3 kinase or AKT inhibitors on the other hand are in progress (K. Flaherty, Massachusetts General Hospital, Boston, MA; M. Davies, UT MD Anderson Cancer Center, Houston, TX; R. Kefford, Westmead Institute for Cancer Research, Westmead, Australia; K. Kim, UT MD Anderson Cancer Center, Houston, TX; D. Schadendorf, University Hospital Essen, Essen, Germany; P. Chapman, Memorial Sloan Kettering Cancer Center, New York, NY).

One of the most worrisome side effects induced by BRAF-V600E inhibitors, particularly by vemurafenib, and less by 436 mutant BRAF inhibitor from Glaxo Smith Kline (GSK), is the induction of squamous cell carcinomas. Notably, combinations of BRAF-V600E inhibitor (vemurafenib or GSK436) with MEK inhibitor (GSK 212 inhibitor or the less potent AZd6244 inhibitor) negated these phenomena (R. Kefford, Westmead Institute for Cancer Research, Westmead, Australia).

Resistance has also been related to up regulation of Hsp90 client proteins, such as PDGFR, mutated N-ras, Cot, cyclin D1, and IGFR (K. Smalley, Moffitt Cancer Center, Tampa, FL). Although Hsp90 inhibitors have been potent inhibitors of V600E positive melanoma cells in vitro, clinical trials with these compounds so far have been disappointing. A novel mechanism of BRAF-V600E resistance is based on epigenetic silencing of genes such as CD82, leading to tumor promotion (G. Robertson, Penn State Univ. College of Medicine, Hershey, PA). Resistance to BRAF-V600E inhibitor may also be explained by induction of autophagy by the inhibitor in melanoma cells which leads to enhanced survival of the cells. Combined treatment of melanoma patients with BRAF-V600E inhibitor and the autophagy inhibitors hydroxy chloroquine and Rapamycin stabilized the disease in 73% of the patients (R. Amaravadi, University of Pennsylvania, Philadelphia, PA).

*Shortly after this conference another mechanism of resistance was published by several of the meeting participants, among others (P.I. Poulikakos et al., Nature 480: 387, 2011). In that study, a subset of cells resistant to BRAF-V600E inhibitor developed variant forms of BRAF-V600E (splicing isoforms with enhanced dimerization) which renders ERK signaling resistant to the inhibitor.

Several laboratories are investigating predictors (biomarkers) of therapeutic responses to BRAF-V600E inhibitors, using in vitro cultures and xenotransplants of melanomas, in efforts to select patients with a high likeliness to respond to therapy (A. Richmond, Vanderbilt-Ingram Cancer Center Nashville, TN; M. Davies, UT MD Anderson Cancer Center, Houston, TX; A. Aplin, Thomas Jefferson University Kimmel Cancer Center). Thus, melanoma patients with N-ras mutations or p10 loss showed decreased survival after BRAF-V600E
targeting. In clinical responders to RAF inhibitors, including BRAF-V600E inhibitor, genes encoding apoptosis, adhesion molecules and Fox D3 were up regulated, whereas cell proliferation and lactase genes were down regulated. In the latter study by A. Richmond, treatment effects most likely were due to targeting wild-type BRAF.

**T cell activation by anti-CTLA4 and Anti-PD1 monoclonal antibodies**

Both CTLA4 and PD1 are molecules that inhibit activated T cells. PD1 also is expressed by exhausted T cells. Upon engagement of these molecules by their respective ligands on tumor cells, T cells become inactivated. Blockade of the receptors on T cells by monoclonal antibodies, ipilimumab (directed to CTLA4) or MDX1106-01 (anti-PD1), may induce T cell activation, ultimately resulting in anti-tumor responses.

Ipilimumab was approved for treatment of metastatic melanoma in 2011. Various clinical centers have reported favorable clinical responses of melanoma patients treated with the antibody as compared to control patients treated with gp100 melanoma peptide vaccine or DTIC chemotherapy (T. Wolchok and P. Chapman, Sloan-Kettering Cancer Center, New York, NY; S. Hodi, Dana Farber Cancer Institute, Boston, MA; A. Ribas, UCLA Medical Center, Los Angeles, CA; J. Weber, Moffitt Cancer Center, Tampa, FLA). Clinical responses of treated patients correlated with an increase in T cells expressing EOMS/CD8, Ki67, ICOS and DR (J. Weber, Moffitt Cancer Center, Tampa, FLA), whereas there was no correlation between the degree of T cell infiltration into tumors and clinical responses (A. Ribas, UCLA Medical Center, Los Angeles, CA).

A prevalent adverse effect of treatment with ipilimumab was colitis, reflecting enhanced T cell activation, leading to autoimmunity and even death in some cases. These serious incidences have become rare due to appropriate medical intervention. Overall, clinical benefits observed with ipilimumab versus standard control treatment were <10% for various response parameters, prompting combination trials of ipilimumab with various immunological and non-immunological agents, such as vaccines, adoptive T cell transfer, cytokines, bevacizumab (Avastin, targets VEGF), Dasatinib (inhibitor of src and other kinases), BRAF-V600E inhibitor (A. Ribas, UCLA Medical Center, Los Angeles, CA; J. Weber, Moffitt Cancer Center, Tampa, FLA; S. Hodi, Dana Farber Cancer Institute, Boston, MA; P. Chapman, Memorial Sloan-Kettering Cancer Center, New York, NY). These trials are ongoing.

Very little research has been done with regard to predictors/biomarkers of immune and clinical responses to therapy with ipilimumab (T. Wolchok, Memorial Sloan-Kettering Cancer Center, New York, NY). Thus, patients with prior
antibodies to NY-ESO1 melanoma-associated antigen seemed to fare better clinically after treatment with ipilimumab.

Blockade of PD1 on T cells may induce superior anti-tumor immune response than blockade of CTLA4 as evidenced by higher clinical response rates seen in melanoma patients treated with anti-PD1 antibody (~33% versus 10-15%; M. Sznol, Yale Cancer Center, New Haven, CT). Anti-PD1 antibody is currently being used as a lymphocyte activator in conjunction with adoptive lymphocyte therapy (J. Weber, Moffitt Cancer Center, Tampa, FLA). This monoclonal antibody was well tolerated and responses were durable (up to 34+ months). Because CTLA4 is up-regulated in T cells after treatment of patients with anti-PD1 antibody, investigators have initiated combination trials with monoclonal antibodies to CTLA4 and PD1 (T. Wolchok, Memorial Sloan-Kettering Cancer Center, New York, NY; J. Weber, Moffitt Cancer Center, Tampa, FLA). However, toxicity enhancement by this combination therapy was considerable.

**Outlook**

Acquired resistance to melanoma therapy with kinase inhibitors, immunostimulatory agents or chemotherapeutic compounds is difficult to overcome by using combination therapies including therapeutics of the same group. Thus, therapeutic resistance developed to a particular kinase may be in part due to activation of another kinase, prompting combination therapies with both kinases. However, chain reactions of resistance development when therapeutics of the same class are being used may prohibit induction of long lasting therapeutic effects. The 2011 SMR meeting gave many examples of interactive treatment modalities based on combinations of drug and immunotherapy. The different mechanisms of these two distinct therapies emphasize their complementarities, raising hopes of longer lasting, more effective therapeutic effects. For example, whereas kinase inhibitors affect preferentially dividing cells and effects are rapid, lymphocytes and antibodies attack both dividing and non-dividing cells and effects often occur delayed. And, importantly, vemurafenib has shown to increase T cell activity (A. Ribas, UCLA Medical Center, Los Angeles, CA), suggesting its combined use with immunomodulators such as vaccines.

This conference focused on the kinase mutant BRAF. C-Kit kinase is another promising target with therapeutic potential for acral lentiginous melanoma, presented by B. Bastian (UCSF Cardiovascular Research Institute, San Francisco, CA) and G. McArthur (Mac Callum Cancer Center, Melbourne, Australia).

Targeting BRAF-V600E with drug or immunotherapy has been hampered by the unavailability of appropriate animal tumor models. Recently, transgenic mouse
models of mutant BRAF have been developed and experimental therapies targeting this molecule \textit{in vivo} have just begun (M. Mc Mahon, UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA; D. Fisher, Massachusetts General Hospital, Boston, MA; M. Bosenberg, Yale University School of Medicine, New Haven, CT).

During the last 2 decades melanoma vaccines have been a major focus of immunotherapeutic approaches for this disease. But all vaccines tested failed in Phase III randomized control trials. It remains to be seen whether initial promising results with the MAGE-3 vaccine will prove significant in Phase III trials (P. Lorigan, University of Manchester, Manchester, England). Focusing the immune response on one tumor antigen may not be sufficient for induction of long lasting tumor regression, although epitope spreading has been a common phenomenon seen in tumor vaccinations. In contrast, broad lymphocyte stimulation by unleashing T cell blockade with antibodies may induce immunity to a large host of tumor antigens which in turn may be therapeutically more effective than initial targeting of a single antigen. The 41BB (CD147) lymphocyte-associated antigen is a novel target to induce T cell activation and expansion in cultures of tumor-infiltrating lymphocytes and will be included in clinical trials soon.