BRAF-inhibition and tumor immune suppression

Shannon M. Steinberg and Mary Jo Turk

1Department of Microbiology and Immunology; Geisel School of Medicine at Dartmouth; Dartmouth-Hitchcock Medical Center; Lebanon, NH USA; 2The Norris-Cotton Cancer Center; Dartmouth-Hitchcock Medical Center; Lebanon, NH USA

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Abbreviations: MDSC, myeloid-derived suppressor cell; TME, tumor microenvironment; Treg, regulatory T cell.

As BRAFV600E-inhibitors become standard treatment for many metastatic melanoma patients, research has begun to elucidate their impact on the tumor immune landscape. Here, we highlight our recent studies demonstrating the ability of melanoma cell-intrinsic BRAFV600E-inhibition to selectively reduce intratumoral immunosuppressive cell populations and enhance antitumor CD8+ T-cell immunity.

Author’s View

As BRAFV600E inhibitors have become a new standard-of-care therapy for metastatic melanoma patients, the effect of BRAF-inhibition on host antitumor immunity has become a crucial field of study. Recent studies have shown that vemurafenib, which can induce rapid regression of BRAFV600E-mutant melanoma, also promotes the effector T-cell composition within the tumor microenvironment (TME). Preclinical studies have thus begun to address the effects of BRAF-inhibition on tumor immune suppression, which is mediated primarily by 2 cell types: regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSCs). Studies in mouse models have recently demonstrated the ability of BRAF-inhibitors to decrease the proportion of Treg in the TME, although there has been substantial disagreement regarding whether BRAF-inhibition also reduces numbers of MDSCs and whether reductions are specific to immunosuppressive cells. It also remains unclear if such immunological changes are secondary effects of inhibitor action on BRAFV600E in melanoma cells themselves (i.e., on-target). Our studies, highlighted herein, recently addressed the downstream consequences of BRAF-inhibition on Treg and MDSC populations within tumors, and the resulting implications for T cell-mediated tumor control.

To answer these questions we selected the Tyr-CreERT BrafCA Ptenlox/lox (Braf/ Pten) model of BRAFV600E-driven melanoma, which we bred onto a C57BL/6 background. Tumors in these mice contained large populations of both MDSCs and Treg, and had a dearth of CD8+ T cells, as has been previously described for human melanomas. Importantly, these tumors underwent stable growth arrest upon treatment with the BRAF-inhibitor PLX4720, a research analog of vemurafenib. This is in contrast to the weak drug sensitivity previously reported for transplantable BRAF-mutant melanoma models. Thus, this autochthonous melanoma may model a subset of aggressive and poorly-immunogenic tumors in patients receiving BRAF-inhibitors.

Our phenotypic analyses of these treated tumors showed that BRAF-inhibition induced a selective loss of immunosuppressive cells from the TME. The most prominent changes was a loss of MDSCs, as evidenced by decreases in both the proportion and total number of CD11b+ Gr-1+ cells. Accordingly, CD11b+ cells isolated from PLX4720-treated tumors were incapable of suppressing T-cell function on a per-cell basis in vitro. In addition to myeloid cells, BRAF-inhibition also significantly decreased the proportion and absolute number of FoxP3+ Treg in tumors. Analysis of untreated size-matched tumors demonstrated that reductions in Tregs and MDSCs were not due to reduced tumor size, but rather that BRAF-inhibition induced a quantitative loss of pre-existent cells from the TME. These changes were not observed in tumor-draining lymph nodes, nor in hosts bearing BRAF-wild-type melanoma, indicating that Treg and MDSC loss were downstream of melanoma cell-intrinsic BRAFV600E-inhibition.

In contrast to the effects of BRAF-inhibition on MDSCs and Treg, the numbers of CD4+ and CD8+ T cell numbers were unchanged by treatment. Thus BRAF-inhibition reduced immunosuppressive cell populations selectively, which is contrary to what has been previously reported for this model. This failure to increase numbers of effector T cells was unexpected in light of clinical observations showing increased CD8+ T cells counts in tumor biopsies upon treatment with vemurafenib. Our adoptive transfer studies with naïve melanoma antigen-specific CD8+ T cells also demonstrated no new T cell priming as a result of PLX4720 treatment. Thus, our data in the Braf/Pten model is consistent with the idea that treatment increases the relative representation of pre-existing CD8+ T cell populations within tumors by selectively eliminating immunosuppressive cells— both MDSCs and Treg (Fig. 1).

Studies in other models have suggested that either CD8+ T cells or CD4+ T cells are absolutely required for BRAF-inhibitor efficacy. In contrast, our own
studies utilizing CD8 and CD4 depleting antibodies, or alternatively RAG1−/− mice, have demonstrated that αβ T cells are not essential for BRAF-inhibitor efficacy against autochthonous Brf/Pten tumors. However, during the period following BRAF-inhibitor treatment, we observed sustained depression of MDSC populations and delayed tumor progression dependent on CD8+ T cells. This implies that CD8+ T cells may provide a redundant mechanism of tumor control in PLX4720-treated mice. It has been suggested that an intermittent vemurafenib dosing schedule may limit the development of drug resistance. Our findings demonstrate that during these “drug holidays,” the patient’s immune response may directly contribute to ongoing disease stability.

The mechanisms by which BRAF-mutant melanoma cells support immunosuppressive cells in the TME remain to be fully defined. Our findings are consistent with the idea that BRAF-inhibition selectively impairs melanoma cell production of Treg and MDSC survival factors. Accordingly, we showed that Treg undergo apoptosis at an increased rate following BRAF-inhibition, although MDSC apoptosis was unchanged. BRAF-inhibition may also block the ability of tumor cells to secrete Treg and MDSC chemoattractants. It has been shown that the C-C chemokine ligand 2 (CCL2), a chemokine associated with recruitment of both Tregs and MDSCs, is decreased in the TME following BRAF-inhibition. Thus immunosuppressive cells may be lost through a combination of factors that modulate recruitment to, and fitness within, the TME.

Work with murine models is beginning to elucidate how dominant oncogenic signaling pathways shape the tumor immune landscape. Routine characterization of molecularly-targeted therapeutics for their impact on the tumor microenvironment may yield surprising information regarding the immunological basis of drug efficacy. These insights will provide a more solid foundation on which to build thoughtful combinatorial therapies that better address patient variability and the heterogeneous and dynamic nature of tumors.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.
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