BRAF MUTATIONS ARE ASSOCIATED WITH T-HELPER CELL INFILTRATION AND POLARIZATION IN MELANOMA

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Background BRAF mutations are highly prevalent in patients with melanoma (50–65%), and mark tumors which are responsive to combination immune checkpoint inhibition (ICI) with nivolumab (α PD-1) and ipilimumab (α CTLA-4). Interestingly, this combination does not improve outcomes over nivolumab alone for patients with BRAF wild-type melanoma.¹ We propose that BRAF mutations shape the immunological response to ICI through tumor derived cytokines and chemokines. While BRAF mutations can influence cytokine production and myeloid cell infiltration, the influence of BRAF mutations on tumor infiltrating CD4+ T-helper cells is underexplored in all tumor types. However, one study described a unique association between BRAF mutant melanoma and signatures of Th17 biology using transcriptomic analysis.² We hypothesize that BRAF mutations create a unique cytokine and chemokine milieu which support the infiltration and polarization of Th17 cells.

Methods To address this hypothesis we employed the congenic BRAF wild-type murine melanoma lines B16F10 and YUMM4.1, and the BRAF mutant line YUMM1.7. These cell lines were implanted subcutaneously into female C57BL/6 mice. Blood cytokines, splenocytes and tumor infiltrating lymphocytes were analyzed at 1 week and 3 weeks post tumor implantation to simulate both early stage and late-stage tumors respectively. Flow cytometry was used to define T-helper cell and myeloid cell phenotypes. In vitro analysis of chemokines and cytokines produced by each cell line were conducted via array and confirmed by ELISA.

Results Th1 and Th17 cells preferentially infiltrated early stage (1 week) BRAF wild-type and mutant tumors respectively while the CD4+ compartment of all tumors was dominated by an overwhelming Treg presence. The CD4+ compartment of endpoint (3 week) B16F10 tumors were almost entirely dominated by Tregs, while YUMM1.7 tumors were infiltrated by a diverse array of T-helper cells. In parallel with these results, we found YUMM1.7 tumors contained more dendritic cells and F4/80hiMHC-II+ macrophages as a percentage of the CD45+ infiltrate. Unique chemokine profiles were associated with each cell line, highlighted by relatively high expression of CXCL10 by BRAF wild-type lines and CXCL12 by both YUMM4.1 and YUMM1.7 cell lines.

Conclusions This work indicates the mutational landscape of melanoma can dynamically impact CD4+ T cell responses to melanoma. This ongoing work is directly translatable and will aid the development of novel cellular therapies tailored to specific immunological phenotypes of patients. Further, this work may help explain disparities in the response to ICI observed between patient populations with defining mutational signatures.

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Ethics Approval All animal studies were conducted under the approval of the Emory Institutional Animal Care and Use Committee and ethical guidelines established by this committee were strictly adhered to.

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