**ABSTRACT**

The mechanism by which defects in interferon (IFN) signaling prevent T cell-mediated antitumor efficacy is unclear. We examined the impact of genetic defects in type I and/or type II IFN pathways on the antitumor efficacy of tumor-specific T cells in the B16 murine model of melanoma. Adoptive cell transfer (ACT) of gp100-specific pmel T cells was effective against B16 tumors (gp100+X) lacking type I or II IFN signaling (p<0.05), but ineffective against tumors lacking both type I and II IFN signaling. We hypothesized that this is due to coupling of MHC-I expression with IFN signaling. Indeed, only tumors lacking both type I and II IFN signaling were unable to augment MHC-I expression in the context of pmel ACT. Augmenting MHC-I expression in tumors lacking type I and II IFN signaling by overexpressing NLRC5 restored the sensitivity to tumor-specific T cells. We phenocopied this effect using a synthetic nanoplex formulation of poly I:C that resensitized tumors to pmel ACT by BO-112 through IFN independent augmentation of MHC-I, likely through induction of NLRC5. Our findings suggest ACT, either alone or in combination with a TLR3/MDA5 agonist, may be an effective approach in tumors lacking IFN signaling.

**METHODS**

- We generated B16 cell lines lacking genes necessary for type I (Ifnar1), type II (Jak2), or type I and II (Jak1) IFN signaling using a CRISPR/Cas9 based approach. We also generated a B16 cell line lacking MHC-I antigen presentation by targeting the B2m gene.
- The pmel model was used to evaluate in vitro and in vivo efficacy of tumor-specific T cells.
- BO-112 is a synthetic nanoplex formulation of poly I:C that activates TLR3, MDA5, and RIG-I, and has shown promising antitumor activity in first-in-human studies.

**RESULTS**

**Figure 4:** In vivo growth of B16 WT and CRISPR modified tumors after adoptive cell transfer (ACT). B16 tumors defective in both type I and II IFN signaling (Jak2KO) do not respond to pmel ACT. B16 tumors defective in only type II (Jak2KO) or only type I (Ifnar1KO) IFN signaling retain sensitivity to pmel ACT, *p < 0.05.

**Figure 5:** B16-Jak2KO tumors are insensitive to tumor-specific T cells due to interferon dependence of MHC-I expression. Pre-treatment of B16 with type I or II IFN is sufficient for cytotoxicity of pmel T cells in vitro, but B16 lacking type I (Ifnar1KO) or type II (Jak2KO) IFN are only sensitive to pmel T cells if pretreated with alternate IFN (A). MHC-I upregulation of B16 is interferon-dependent (B). B16 wildtype and Ifnar1KO tumors, but not others, upregulate MHC-I in vivo (C). Five days after pmel ACT and IL-2, B16-Jak2KO tumors, but not B16-Jak1KO or B16-B2mKO tumors, upregulate MHC-I expression (C, right).

**Figure 6:** NLRC5 restores sensitivity of B16-Jak2KO tumors to tumor-specific T cells by augmenting MHC-I expression through an interferon-independent mechanism.

**Figure 7:** Intratumoral (i.t.) BO-112 restores sensitivity of B16-Jak1KO tumors to ACT in vivo. B16 JAK1KO tumors were injected i.t. with BO-112 on days 1 and 4 after ACT (A). Tumors treated with pmel ACT and BO-112, were enriched for genes involved with T cell cytotoxicity, MHC-I presentation, and T cell activation (B), *p < 0.05.

**CONCLUSIONS & DISCUSSION**

- Adoptive cell therapy (ACT) alone is effective in tumors that are resistant to immune checkpoint blockade due to tumor intrinsic defects in type II IFN signaling.
- However, B16-Jak1KO melanoma lacking type I and II interferon signaling does not respond to ACT due to interferon-dependent MHC-I antigen presentation.
- Uncoupling antigen presentation from interferon signaling by overexpressing NLRC5 restores efficacy of ACT in B16-Jak1KO melanoma.
- Intratumoral BO-112 results in IFN-independent (and dependent) upregulation of MHC-I, thus pharmacologically uncoupling MHC-I expression from IFN signaling.
- BO-112 restores the efficacy of ACT in B16-Jak1KO melanoma in MHC-I dependent manner, through interferon-independent induction of NLRC5.

**ACKNOWLEDGMENTS**

Research was funded by the Parker Institute for Cancer Immunotherapy. Flow cytometry was performed at the Eli & Edythe Broad Center for Regenerative Medicine & Stem Cell Research. Murine studies were performed through DLAM in the Radiation Oncology Vivarium. A. Kalbasi is supported by a UCLA CTSI KL2 Award.

**REFERENCES**