Targeting Immune Suppressive Myeloid Cell Pathways for the Treatment of Cancer

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Introduction

- The tumor microenvironment is populated by a variety of myeloid cell subsets (Figure 1, left) that contribute to maintaining an immunosuppressive tumor microenvironment.
- Increased myeloid cell infiltration in tumors is associated with reduced survival and reduced responses to immunotherapy in several types of cancers.
- Myeloid cells mediate their immunosuppressive effects via multiple mechanisms, including expression of immune checkpoint protein ligands, activation of the PI3Kγ pathway, and release of arginase-1 (ARG1) (Figure 1, right).
- The significant role of myeloid cells in dampening anti-tumor immunity makes them an attractive target for immunotherapy. To that end, we have evaluated the effects of potent and selective inhibitors of ARG1 and PI3Kγ:
  - AB474: Inhibits ARG1, an arginine-depleting enzyme that suppresses T cell responses.
  - A0305137: Inhibits PI3Kγ, a phosphatidylinositol kinase predominantly involved in suppressing pro-inflammatory responses from myeloid cells.

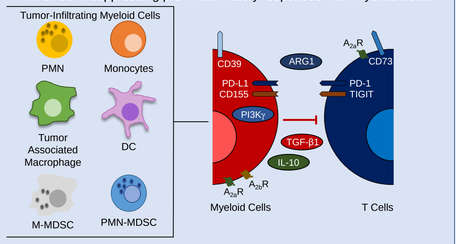


Figure 1. Various myeloid cell subsets are present in tumors (left) and can inhibit productive T cell responses through various mechanisms (right).

Methods

- The ability of AB474 to rescue recombinant ARG1-mediated inhibition of T cell activation was assessed in human T cells isolated from healthy donors.
- Anti-tumor activity was determined using the MCA205 syngeneic tumor model.
- Monocyte-derived dendritic cells (moDC) were differentiated from CD14$^+$ monocytes with IL-4 and GM-CSF.
- Macrophages were differentiated from CD14$^+$ monocytes with M-CSF. Macrophages were then polarized into M1 macrophages with LPS+IFN-γ or M2 macrophages with IL-4 or IL-10 in the presence or absence of A0305137. RNA was then collected for gene expression analysis.
- CD14$^+$ monocytes from healthy donors were stimulated with LPS+IFN-γ +/- A0305137. Supernatant was collected for detection of IL-12 or RNA was isolated for gene expression analysis.
- Additionally, M1 macrophages +/- A0305137 were placed in a mixed lymphocyte reaction (MLR) with CD4$^+$ T cells in the presence or absence of AB122, a PD-1 blocking antibody, or an IL-12 blocking antibody.
- To determine the ability of A0305137 to inhibit phosphorylation of Akt (pAkt), a signaling molecule downstream of PI3Kγ activation, human whole blood was stimulated with CXCL12. pAKT was measured by flow cytometry.

Results

ARG1 is Expressed in Suppressive Immune Cells

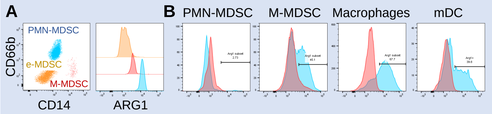


Figure 2. A) ARG1 expression was measured in human whole blood MDSC subsets by flow cytometry. B) Mouse ARG1 expression was measured by flow cytometry in MCA205 tumors. Red, isotype; blue, stain.

AB474 Rescues ARG1-mediated Inhibition of CD8$^+$ T Cell Activation

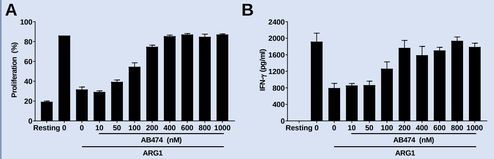


Figure 3. CD8$^+$ T cells were activated in the presence of recombinant ARG1 +/- AB474. AB474 prevents ARG1-mediated inhibition of T cell A) proliferation and B) IFN-γ production.

AB474 Acts in Combination with anti-PD-1 to Reduce Tumor Growth

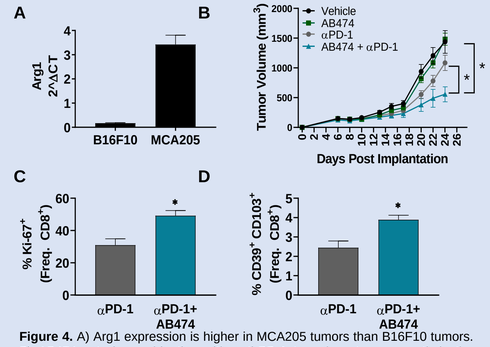


Figure 4. A) Arg1 expression is higher in MCA205 tumors than B16F10 tumors. B) AB474 acts in combination with anti-PD-1 to reduce tumor growth in the MCA205 model. B) AB474 increases CD8$^+$ T cell C) proliferation and the D) frequency of CD39$^+$CD103$^+$ CD8$^+$ T cells in tumor infiltrating lymphocytes compared to anti-PD-1 alone. *, p<0.05

PI3Kγ is Expressed in Myeloid Cell Subsets

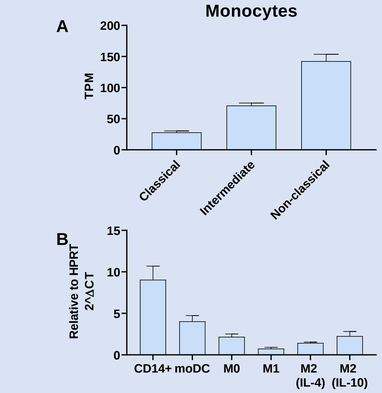
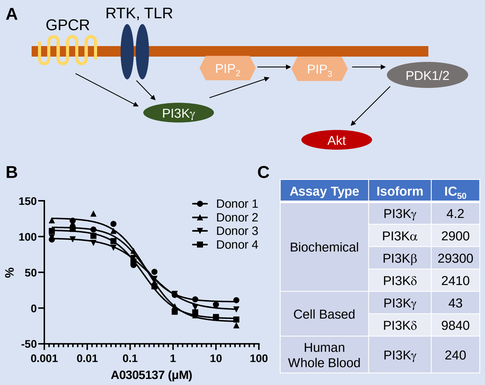


Figure 5. A) PIK3CG expression derived from RNAseq of sorted peripheral immune cells from healthy donors (Monaco G., et al. Cell Reports. 2019). B) PIK3CG expression in monocytes and in *in vitro* differentiated moDC and macrophages and polarized macrophages.

A0305137 Suppresses Downstream PI3Kγ Signaling



Assay Type	Isoform	IC_{50}
Biochemical	PI3Kγ	4.2
	PI3Kα	2900
	PI3Kβ	29300
	PI3Kδ	2410
Cell Based	PI3Kγ	43
	PI3Kδ	9840
Human Whole Blood	PI3Kγ	240

Figure 6. A) Receptor stimulation activates PI3Kγ signaling leading to phosphorylation of Akt. B) A0305137 inhibits CXCL2-mediated phosphorylation of Akt in blood CD14$^+$ monocytes as determined by phospho-flow cytometry. C) A0305137 is a potent and selective PI3Kγ inhibitor.

PI3Kγ Inhibition Increases IL-12 Production from Monocytes

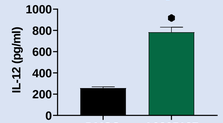


Figure 7. A0305137 increases IL-12 production from CD14$^+$ monocytes stimulated with LPS+IFN-γ. *, p<0.05

PI3Kγ Inhibition Increases Pro-inflammatory Cytokine Expression

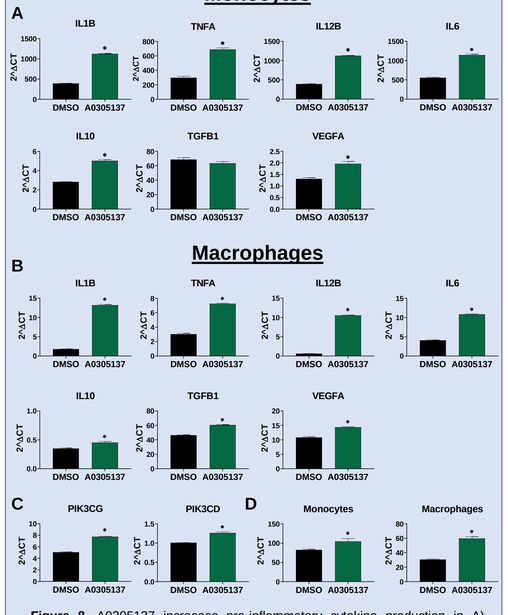


Figure 8. A0305137 increases pro-inflammatory cytokine production in A) monocytes and B) macrophages after LPS+IFN-γ stimulation. C) A0305137 increases expression of PIK3CG in M1 macrophages. D) A0305137 increases ADORA2A ($A_{2a}R$) expression in LPS+IFN-γ stimulated monocytes and macrophages. *, p<0.05

PI3Kγ Inhibition Increases Pro-inflammatory Cytokine Expression in M2 Macrophages

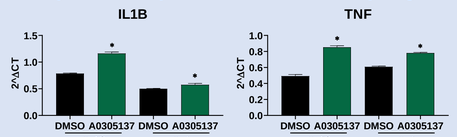


Figure 9. A0305137 increases IL1B and TNFA expression in IL-4 and IL-10 polarized M2 macrophages. *, p<0.05

PI3Kγ Inhibition Increases M1 Macrophage T Cell Activation Capacity

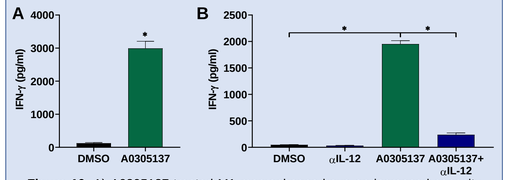


Figure 10. A) A0305137-treated M1 macrophages have an increased capacity to activate CD4$^+$ T cells in an MLR, in an IL-12-dependent manner (B). *, p<0.05

PI3Kγ Inhibition Acts in Combination with PD-1 Blockade to Increase T Cell Activation

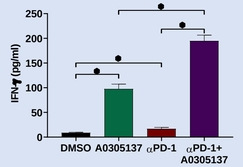
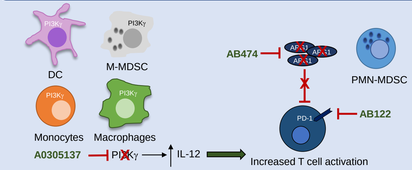


Figure 11. A0305137-treated M1 macrophages and anti-PD-1 (AB122) lead to enhanced T cell activation in an MLR. *, p<0.05

Conclusions



Targeting indirect (PI3Kγ) or direct (ARG1) mechanisms of immunosuppressive myeloid cells relieves T cell suppression, resulting in effective T cell responses.