Amezalpat, a Peroxisome Proliferator-Activated Receptor Alpha (PPARα) Antagonist, Inhibits Suppressive Immune Cell Development, Activation and Function.

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Abstract #2171

OBJECTIVE

Characterize the impact of amezalpat-induced inhibition of PPARα on suppressive immune cell function.

BACKGROUND

Peroxisome Proliferator-Activated Receptor α (PPARα):

- Nuclear hormone receptor that functions as master regulator of fatty acid oxidation (FAO), upregulated in multiple cancers, and expressed by immune cells[1]
- Inhibition of PPARα may preferentially target both FAO-reliant tumor cells and suppressive immune cells such as tumor-associated macrophages and T regulatory cells [2]

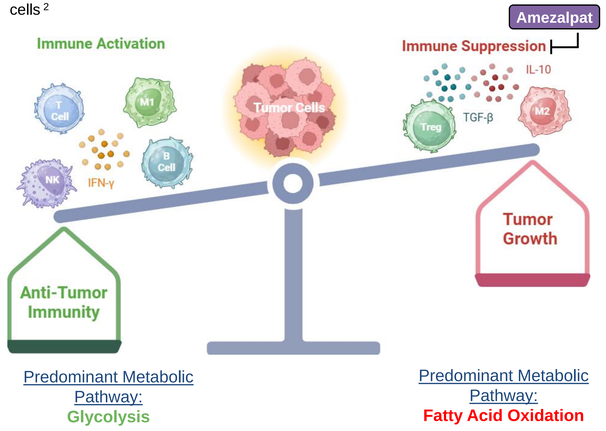

Figure 1 | Putative role of amezalpat in immune-mediated anti-tumor immunity.

Amezalpat:

- First-in-class, orally-bioavailable, potent, highly selective competitive antagonist of PPARα
- Hypothesized to block tumor-promoting FAO in both tumor and immune cells to alleviate PPARα-induced immune suppression
- Clinical experience:
  - ***Phase 1 trial in advanced solid tumors (NCT03829436):*** 53% disease control rate as single agent and 30% objective response rate (ORR) in combination with nivolumab at the two highest amezalpat doses tested[3]
  - ***Phase 1b/2 randomized trial in 1L metastatic HCC (NCT04524871):*** 30% confirmed ORR with amezalpat plus atezolizumab + bevacizumab vs. 13.3% with atezolizumab + bevacizumab alone; median OS of amezalpat plus atezolizumab + bevacizumab arm: 21.2 months (15.4, NE) vs. 15.0 months (10.5, 18.5) for atezolizumab + bevacizumab arm; HR: 0.65 (0.35, 1.21). Data cut-off: 14 Feb 2024.
  - Tolerable safety profile alone and in combination w/ nivolumab or atezolizumab + bevacizumab[3,4]

METHODS

***Ex Vivo* Immunosuppressive (M2) Macrophage and T regulatory (Treg) cell differentiation**

- Primary monocytes or naïve CD4⁺ T cells were isolated from human PBMCs by EasySep™ magnetic isolation
- CellXVivo™ polarization kits for immunosuppressive (M2) (CDK103) and Treg (CDK006) polarization *ex vivo* were performed according to the manufacturer's instructions

Tumor:Immune Cell Co-Cultures

- Either M2 (6 days) or Treg (5 days) cells were differentiated as described above. Donor-matched CD8⁺ T cells were stimulated for 24h prior to addition
- Tumor cells (SNU-449 or 769-P) were combined with either M2 or Tregs and CD8⁺ T cells. Co-cultures were then incubated for 3-5 days prior to endpoint analysis

ELISA, Luminex®, and Flow Cytometry

- Flow cytometry was performed using a LSRFortessa™ for M2 and Treg cell markers
- Cell supernatant was removed on final day of co-culture and analyzed using Luminex or ELISA (immunoreactive TGF-β)

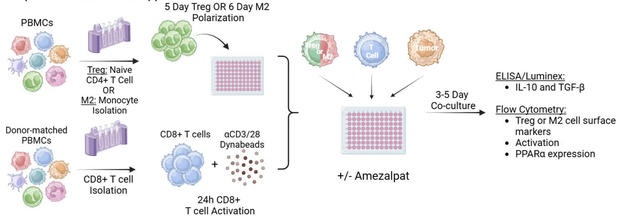

Figure 2 | Tumor:Immune cell co-culture experimental design with immunosuppressive (M2) or Tregs.

RESULTS

Amezalpat decreases immunosuppressive macrophage differentiation and mitochondrial capacity.

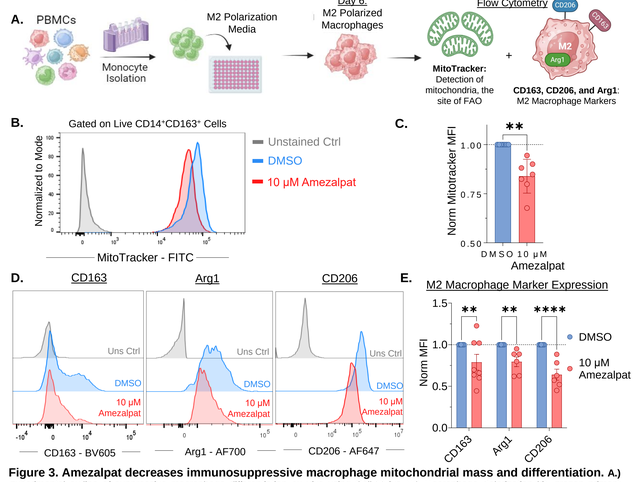

Figure 3. Amezalpat decreases immunosuppressive macrophage mitochondrial mass and differentiation. A.) Experimental outline of suppressive macrophage differentiation *ex vivo* using Cell XVivo M2 Macrophage Polarization kit. Frozen primary peripheral blood mononuclear cells (PBMCs) were put through magnetic StemCell monocyte isolation. Isolated monocytes were then differentiated for 6 days in M2 Macrophage Polarization media. On day 3 of polarization, media was refreshed, and cells were dosed with 10 μM amezalpat in DMSO vs. DMSO alone in the presence of 30 μM oleic acid. On day 6, differentiated macrophages were analyzed by flow cytometry for mitochondrial mass with Mitotracker and suppressive macrophage markers (CD163, Arg1, and CD206). **B.)** Representative histogram of MitoTracker staining in differentiated macrophages on day 6 of unstained control (gray), DMSO treatment (blue), and 10 μM amezalpat treatment (red). **C.)** Bar graph of MitoTracker mean fluorescence intensity (MFI) normalized to DMSO of each individual donor (n=7 donors). **D.)** Representative histograms of CD163 (left), Arg1 (center), CD206 (right) in differentiated macrophages on day 6. **E.)** Bar graph of MFI normalized to DMSO of CD163 (n=8), Arg1 (n=6), and CD206 (n=6). Two-tailed Student's T-Test, **:p<0.01; ****:p<0.0001.

RESULTS (cont.)

Amezalpat decreases anti-inflammatory cytokine production in immune cell and tumor cell co-cultures.

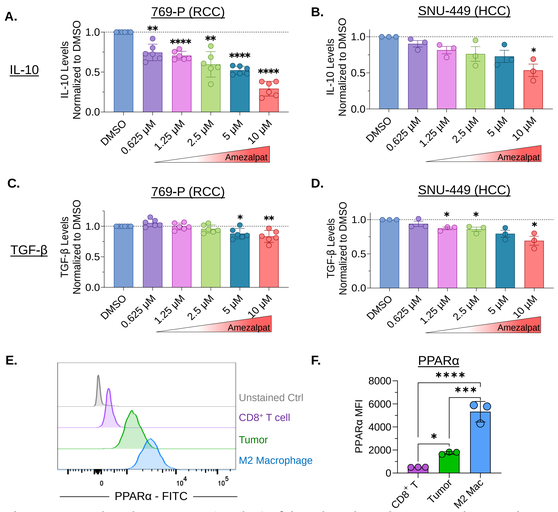

Figure 4. Amezalpat decreases IL-10 and TGF-β in a dose-dependent manner in tumor:immune cell co-cultures. Tumor:Immune cells were co-cultured as detailed in Figure 2. Supernatants of tumor:immune cell co-cultures were analyzed by Luminex for IL-10 in **A.)** 769-P (n=1) and **B.)** SNU-449 (n=3) cell lines treated with increasing amounts of amezalpat in DMSO vs. DMSO control alone. Supernatants were also analyzed for TGF-β by ELISA in **C.)** 769-P (n=1) and **D.)** SNU-449 (n=3) cell lines with increasing treatment of amezalpat. Co-cultures were analyzed via flow cytometry for CD8+ T cell, Tumor, and M2 macrophage markers and PPARα expression. **E.)** Representative histogram and **F.)** quantification of PPARα expression in within CD8+ T cells (purple; CD45⁺CD3⁺ CD8⁺), tumor (green; CD45⁻CD3⁻), and M2 macrophages (blue; CD45⁺ CD3⁻CD14⁺ CD163⁺) (n=3). (A-D) One-way ANOVA of all conditions vs. DMSO. *:p<0.05; **:p<0.01, ****:p<0.0001. F.) One-way ANOVA with multiple comparisons between cell types.

Amezalpat decreases T regulatory cell differentiation and activation in the presence of tumor cells and CD8⁺ effector T cells.

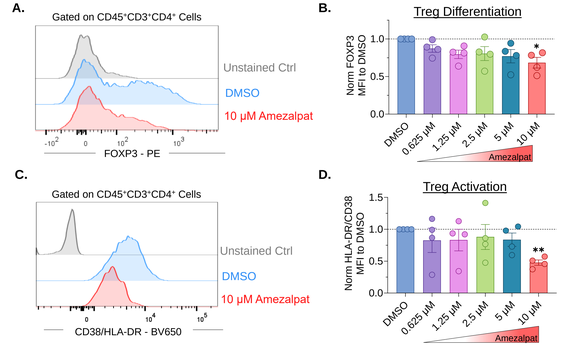

Figure 5. Amezalpat induces a significant decrease in Treg differentiation (FOXP3) and activation (HLA-DR/CD38). Tumor (SNU-449):Immune cells were co-cultured as detailed in Figure 2. Flow cytometry was performed on co-cultures to determine Treg differentiation (FOXP3) in response to DMSO or amezalpat treatment in increasing doses as shown by **A).** representative histograms and **B.)** quantification of FOXP3 MFI. Additionally, HLA-DR/CD38 expression was detected in response to amezalpat treatment via flow cytometry as shown by **C).** representative histograms and **D.)** quantification of HLA-DR/CD38 MFI. N=4 individual donors; One-way ANOVA of all conditions vs. DMSO. *:p<0.05; **:p<0.01.

Amezalpat promotes a pro-inflammatory environment by decreasing immunosuppressive cytokine levels.

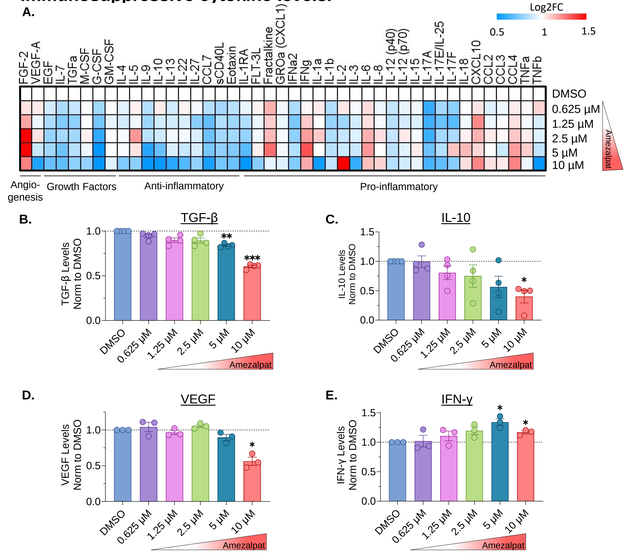

Figure 6. Amezalpat decreases immunosuppressive cytokine production in a tumor:treg co-culture. Tumor (SNU-449) and Treg cells were co-cultured as detailed in Figure 2. Luminex was performed to identify cytokines present in the supernatants of the tumor:treg co-culture in response to increasing doses of amezalpat treatment vs. DMSO control alone. **A).** Heatmap of Log2FC of measured cytokine levels in supernatant of co-cultures normalized to DMSO. **B.)** ELISA was performed on supernatants to identify immunoreactive TGF-β levels. Quantification of Luminex data of **C.)** IL-10, **D.)** VEGF, and **E.)** IFN-γ, normalized to DMSO. N=4 individual donors; One-way ANOVA of all conditions vs. DMSO. *:p<0.05; **:p<0.01; ***:p<0.001.

CONCLUSIONS

- Amezalpat is a potent competitive antagonist of PPARα, a key transcriptional regulator of FAO, and induces immune activation consistent with one aspect of its proposed mechanism of action:
  - Amezalpat treatment of immunosuppressive (M2) macrophages:
    - Decreases M2 macrophage differentiation and mitochondrial mass.
    - Reduces anti-inflammatory cytokine levels (IL-10 &TGF-β).
  - Amezalpat treatment of T regulatory cells:
    - Reduces anti-inflammatory cytokine levels (IL-10, TGF-β, VEG-F) and increases pro-inflammatory IFN-γ.
    - Decreases Treg differentiation (FOXP3 expression) and activation (HLA-DR/CD38 expression).
- Overall, amezalpat decreases immunosuppressive T cells and macrophage function and differentiation to promote a pro-inflammatory anti-tumor response.

REFERENCES: 1. Bougarne N, et al. *Endocr Rev.* 2018;39:760–802. **2.** Zhang, S. et.al. *Cell Death Discov.* 2024; **3.** Yarchoan M, et al. *J Clin Oncol.* 2022; 40(16): Abstract 3005; **4.** Tempest Therapeutics Press Release. October 11, 2023. https://ir.tempesttx.com/news-releases/news-release-details/tempest-releases-new-data-demonstrating-superiority-tpst-1120.

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