Blocking Adenosine Production with ORIC-533, a CD73 Inhibitor with Best-In-Class Properties, Reverses Immunosuppression in High-AMP Environments

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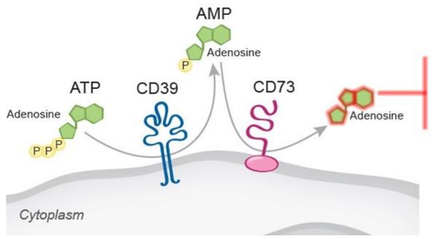
CD73 Mediates Immunosuppression and Therapeutic Resistance via Adenosine Production



- T cell priming
- T cell expansion
- T cell activation / cytolytic activity
- NK degranulation
- Macrophage M1 polarization
- DC maturation / activation

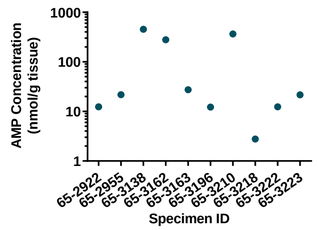
1. ORIC-533 is a Highly Potent and Selective Inhibitor of CD73

Enzyme	IC_{50} [µM]
CD73	0.000089
CD39/ENTPD1	>50
CD39L3/ENTPD3	>50
CD39L4/ENTPD5	>50
ENPP1	>50
ENPP7	>50
SAHH	>50
PDE1a, PDE1b, PDE1c	>50
PDE3a	>50
PDE4a	>50
PDE7a, PDE7b	>50
PDE9a	>50
ACPP	>50
TNAP	>50
NT5C2	>50
NT5CB3	28.1
NT5M	>50

Figure 1. ORIC-533 has an IC_{50} of 89 pM ± 20 pM (n=12) against CD73 as measured by malachite green detection system. Biochemical selectivity profile of ORIC-533 against 19 representative enzymes encompassing families of 5'-nucleotidases, ectonucleotidases, ecto-nucleotide pyrophosphatases/phosphodiesterases, phosphodiesterases and an adenosyl homocysteinase. Average IC_{50} displayed (n=2)

2. ORIC-533 is Active in a High AMP Environment

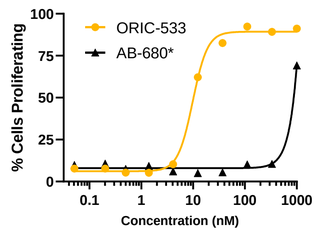
A. AMP in Human Primary CRC Tumors



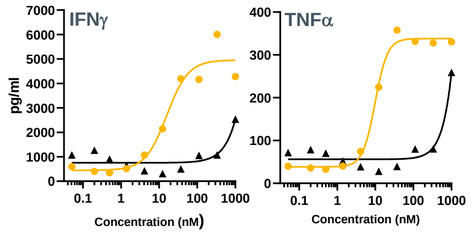
AMP levels in primary tumors reach up to 500µM

Figure 2. A. AMP levels determined by LC-MS/MS in primary CRC tumor samples. **B.** Human PBMC-derived $CD8^+$ T cells activated for 24hr with tetrameric anti-CD3/CD28/CD2 antibodies in serum-free media, labeled with CellTrace Violet. Compounds and AMP were added at indicated concentrations and cells were incubated for 96 hrs and quantified by flow cytometry. **C.** Cytokines in cell supernatants were measured by MSD ELISA. * Bowman et al., 2019

B. $CD8^+$ T Cell Proliferation at 1mM AMP

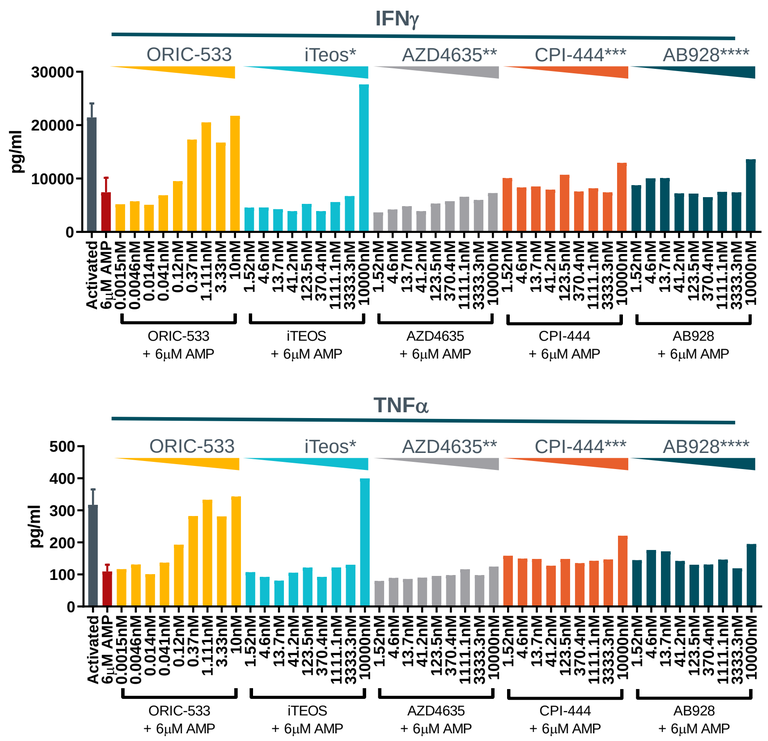


C. Cytokine Production at 1mM AMP

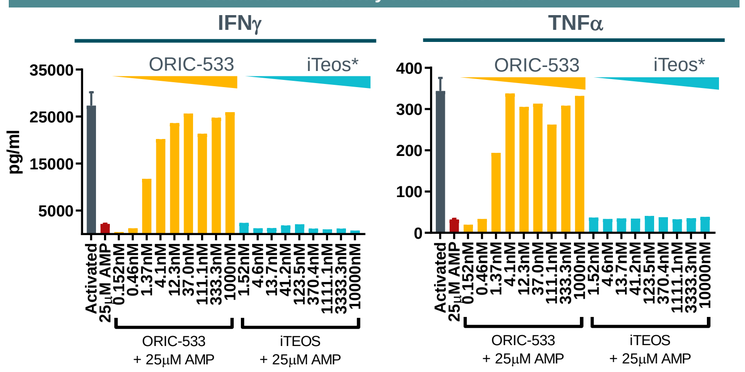


3. ORIC-533, But Not A2R Antagonists, Rescues $CD8^+$ Cytokine Secretion in Moderate AMP

ORIC-533 Potently Rescues $CD8^+$ Cytokine Production at 6 µM AMP



ORIC-533 Potently Rescues $CD8^+$ Cytokine Production at 25 µM AMP Level Which is Commonly Found in Tumors

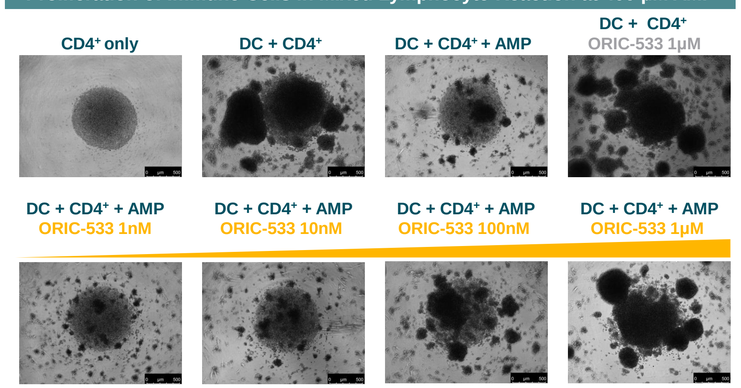


Some A2R antagonists rescue CD8+ T cell function, but only in low micromolar AMP level, using highest dose tested

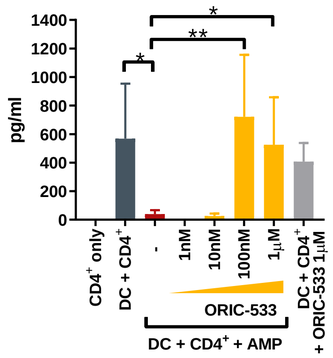
Figure 3. Human $CD8^+$ T cell cytokine assessment performed as described in Figure 2, with either 6 or 25 µM AMP added to the media with indicated compounds.*WO2020065036A1 Compound 8b; **Borodovsky et al., 2017; ***Willingham et al., 2016; ****Seitz et al., 2019

4. ORIC-533 Rescues Immune Cell Activation in Mixed Lymphocyte Reaction in High AMP

Proliferation of Immune Cells in Mixed Lymphocyte Reaction at 400 µM AMP



IFNγ Production at Day 4



IFNγ Production at Day 6

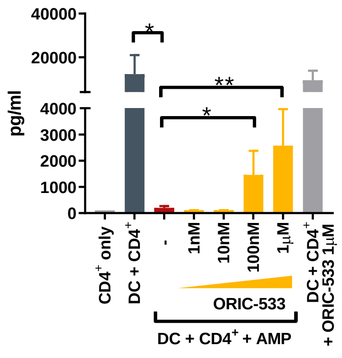


Figure 4. Human allogeneic immature dendritic cells (DC) and $CD4^+$ T-cells were plated onto 96-well plates at 1:2 ratio and treated with indicated concentrations of ORIC-533 followed by addition of 400 µM AMP. Each condition was performed in quintuplicate. Representative images of wells were taken after 6 days of co-culture with an inverted microscope at 5X magnification. Scale bar: 500 µm. IFNγ was measured in cell supernatants by MSD ELISA after 4 and 6 days of co-culture. t-test: **p-value <0.01, *p-value <0.05

CONCLUSIONS

ORIC-533 is a novel CD73 inhibitor with potential best-in-class properties in reversing immunosuppression with:

- picomolar potency and high selectivity in biochemical assays
- evidence of complete $CD8^+$ T cell functional rescue at low nanomolar concentrations across a range of AMP environments from low to high AMP (6µM-1mM AMP)
- the ability to revert immunosuppression in elevated AMP contexts found in the tumor microenvironment, in contrast to adenosine receptor antagonists and other CD73 inhibitors
- the capacity to rescue activation of AMP-suppressed $CD4^+$ T cells co-cultured with allogeneic immature dendritic cells

ORIC-533 IND filing anticipated in first half of 2021

AACR Annual Meeting, April 10-15, 2021

Abstract #LB-163