CD73 Inhibition with a Novel Orally Bioavailable Small Molecule Blocks Adenosine Production and Rescues T-cell Activation

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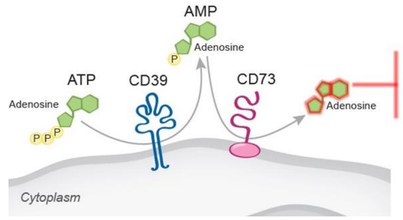
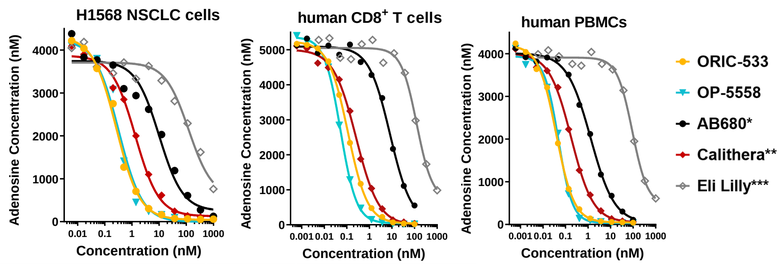
CD73 mediates immunosuppression and therapeutic resistance via adenosine production



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

1. ORIC's novel CD73 inhibitors potently suppress adenosine production from AMP



Compound	Biochem	H1568	CD8+
	IC_{50} (nM)	EC_{50} (nM)	EC_{50} (nM)
ORIC-533 clinical candidate	0.1	0.1	0.1
OP-5558 tool compound	0.1	0.1	0.05
AB680*	0.8	5.3	5
Calithera**	0.2	1.3	0.2
Eli Lilly***	48	88	125

Figure 1. Cells were pre-treated with compounds for 15min, followed by addition of 10uM AMP/5uM EHNA for 1hr. Adenosine in supernatant was quantified by LC-MS/MS
* Bowman *et al*, 2019
** WO2019246403A1 Compound 9
*** WO2019168744A1 Example 2

2. ORIC-533 and OP-5558 exhibit slow dissociation rate and sustained effects after washout

ORIC inhibitors bind CD73 with high affinity and exhibit slow dissociation rate by SPR

CD73 inhibitor	K_D	K_{ON}	K_{OFF}
ORIC-533	30 pM	$1.60*10^6$ $M^{-1}s^{-1}$	$4.84*10^{-5}$ s^{-1}
OP-5558	64 pM	$1.25*10^6$ $M^{-1}s^{-1}$	$8.00*10^{-5}$ s^{-1}
AB680	143 pM	$1.24*10^6$ $M^{-1}s^{-1}$	$1.78*10^{-4}$ s^{-1}

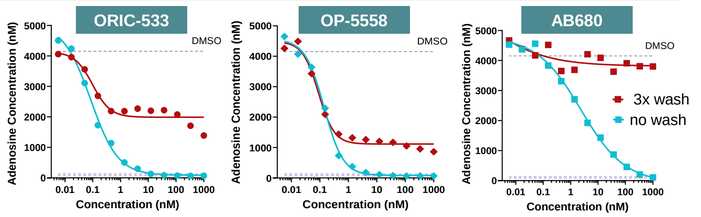


Figure 2. Adenosine generation in supernatants of H1568 cells, quantified by LC-MS/MS. **No wash:** cells pre-treated with compounds for 15min, followed by addition of 10uMAMP/5uM EHNA for 1hr. **3x wash:** cells treated with compounds for 1hr, washed with media 3 times, replenished with fresh media and AMP/EHNA added after compound wash-out and adenosine quantified after 1 hr. SPR, surface plasmon resonance.

3. ORIC-533 rescues activation of CD8+ T-cells exposed to AMP

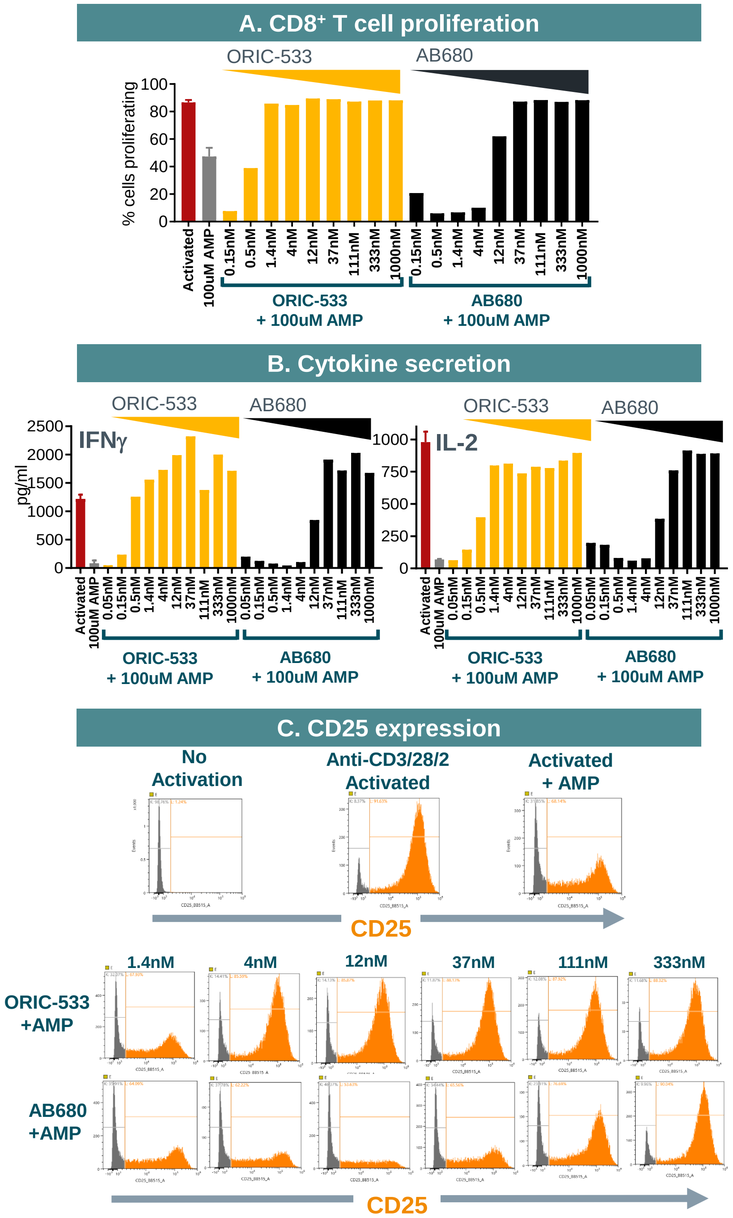
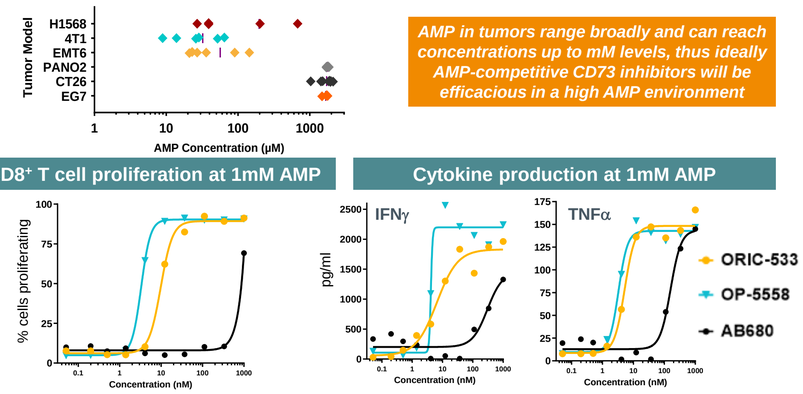


Figure 3. Human PBMC-derived $CD8^+$ T cells were activated for 24hr with tetrameric anti-CD3/CD28/CD2 antibodies in serum-free media, labeled with CellTrace Violet and plated onto 96-well plates. Compounds and AMP were added at indicated concentrations and cells were incubated for 72-96hrs. **A.** Proliferating cells were quantified by flow cytometry. **B.** Cytokines in cell supernatants were measured by MSD ELISA. **C.** CD25 expression on live cells was assessed by flow cytometry.

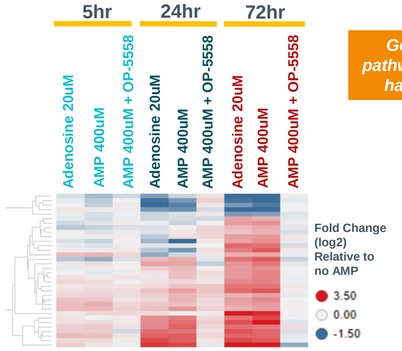
4. ORIC's potent AMP-competitive inhibitors are active in a high AMP environment



AMP in tumors range broadly and can reach concentrations up to mM levels, thus ideally AMP-competitive CD73 inhibitors will be efficacious in a high AMP environment

Figure 4. AMP levels determined by LC-MS/MS in homogenized subcutaneous tumor tissues of individual mice. Human CD8+ T cell proliferation and cytokine assessment performed as described in prior figure.

5. CD73 inhibition efficiently restores AMP-induced transcriptional changes



Gene signature indicative of adenosine pathway activation may help identify patients harboring elevated adenosine signaling

Figure 5. Transcriptional changes of genes comprising the adenosine activation signature in CD8+ T cells in a representative donor (based on three independent donors); assessed using NanoString technology; OP-5558 at 100nM.

CONCLUSIONS

ORIC-533 is a novel CD73 inhibitor with best-in-class properties

- Potently blocks adenosine production from AMP
- Has slow dissociation rate / long residency time on CD73 protein
- Continues to inhibit adenosine production after drug washout
- Rescues activation of CD8+ T cells exposed to AMP
- Potent in high AMP [1mM] environment

See also: AACR 2020 Poster #10268 An Orally Bioavailable Inhibitor of CD73 Reverts Intratumoral Immunosuppression and Promotes Anti-tumor Response

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