An Orally Bioavailable Inhibitor of CD73 Reverts Intratumoral Immunosuppression and Promotes Anti-Tumor Response

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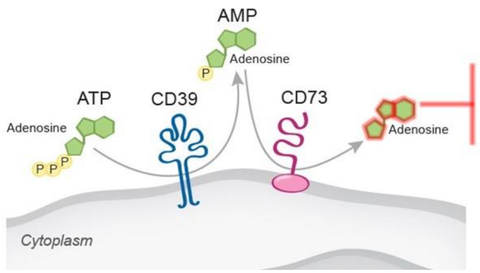
BACKGROUND

CD73 (ecto-5'-nucleotidase):

- Is required for adenosine (ADO) production and linked to therapy resistance
- Is overexpressed across cancer types driving local elevation of ADO
- Overexpression is correlated with poor prognosis
- Mediates immunosuppression and chemoresistance via ADO production

1. ADO signaling is broadly immunosuppressive



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

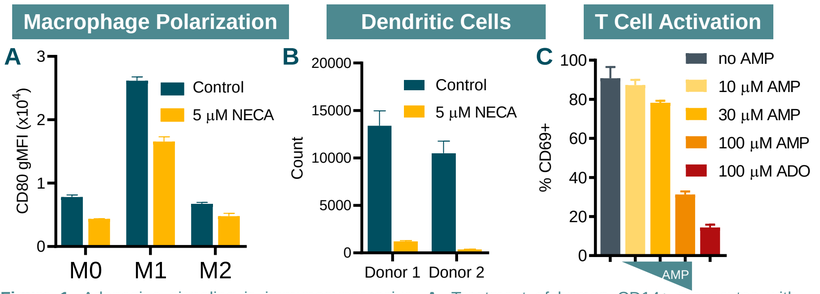


Figure 1. Adenosine signaling is immunosuppressive. **A.** Treatment of human CD14+ monocytes with adenosine (ADO) analog, NECA, 5 μM for 5d, then M-CSF (M0), M-CSF+LPS+IFNγ (M1), or M-CSF+IL-4 (M2) +/- 5 μM NECA for 1d impaired macrophage polarization. **B.** Treatment of DCs from C57BL/6 bone marrow with NECA also reduced rmFlt3L/rmGM-CSF-mediated DC proliferation. **C.** CD73-mediated generation of ADO from AMP reduced Balb/c T cell activation by anti-CD3 antibody treatment + 50 μM EHNA +/- AMP or ADO for 1d. Mean and SD are shown.

2. Discovery of potent and orally bioavailable CD73 inhibitors

Compound	Biochem	Murine CD8+	EMT6	Mouse PO (150 mg/kg)		
	IC_{50} (nM)	EC_{50} (nM)	EC_{50} (nM)	$AUC_{0\text{-last}}$ (μM*h)	C_{max} (μM)	$t_{1/2}$ (hr)
AB680*	0.8	21	210	IV formulation in clinical trial		
OP-5558	0.1	0.1	0.25	55.9	39.3	2.88
ORIC-533 clinical candidate	0.1	0.3	1.1	103	49.8	2.98

*Bowman C.E. *et al*, Biochemistry 2019, 58, 3331-3334.

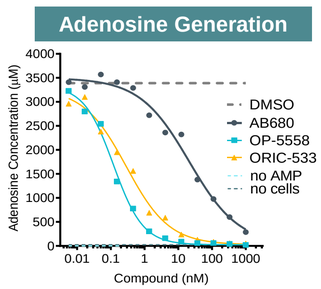


Figure 2. OP-5558 and ORIC-533 have greater oral bioavailability than a clinical-stage CD73 inhibitor. OP-5558 and ORIC-533 effectively inhibit in vitro adenosine generation from AMP in C57BL/6 CD8+ T cells at nM concentrations, as compared to a clinical-stage CD73 inhibitor (right panel).

3. ORIC CD73 inhibitors rescue AMP-mediated CD8+ T cell suppression

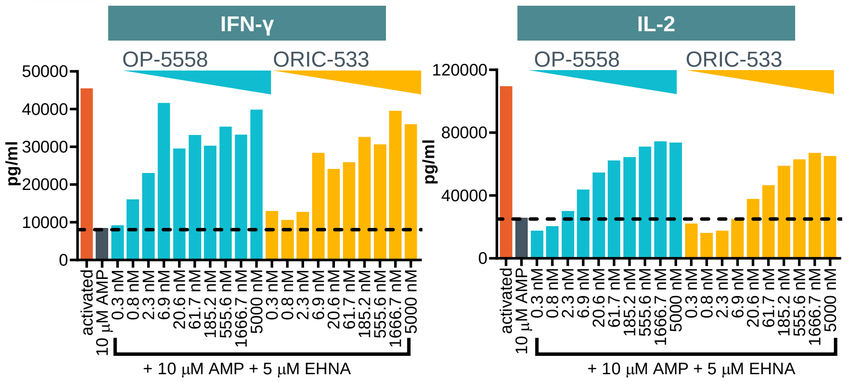


Figure 3. CD8+ T cells isolated from C57BL/6 spleens were activated with CD3/CD28 Dynabeads, then treated with CD73 inhibitors and 10 μM AMP + 5 μM EHNA for 24hr. Cytokines in cell supernatants were measured by MSD ELISA. Dashed lines indicate cytokine secretion in the presence of AMP, without CD73 inhibitors.

4. Oral dosing of OP-5558 and ORIC-533 significantly inhibit tumor growth and reduce intratumoral ADO

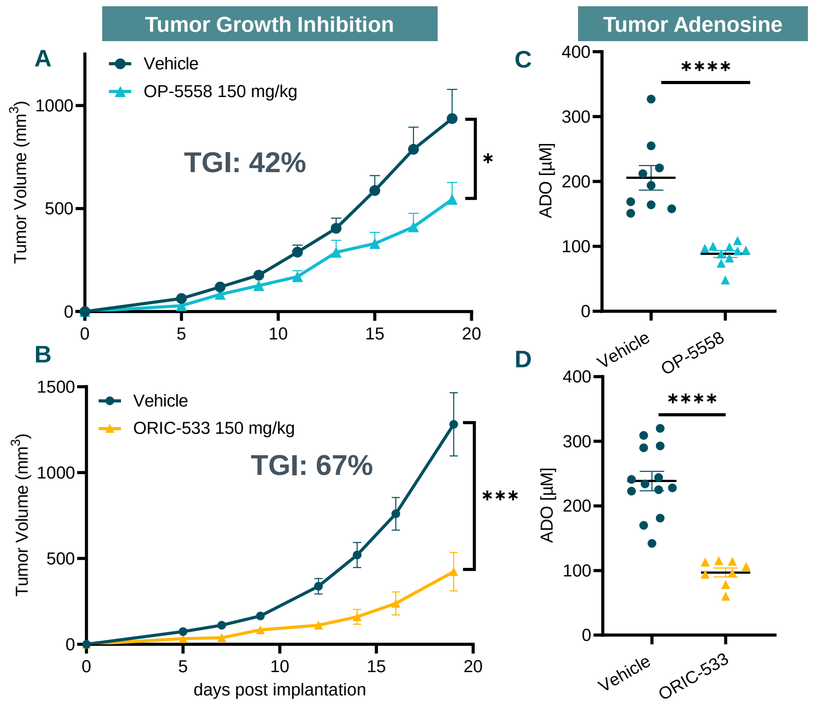


Figure 4. E.G7-OVA mice were orally dosed with **A.** OP-5558 and **B.** ORIC-533, PO QD for 19d at 150 mg/kg. Intratumoral adenosine was also significantly reduced both with **C.** OP-5558 and **D.** ORIC-533, as assessed in terminal tumor. Mean and SEM are shown; *p<0.05; ***p<0.001; ****p<0.0001; t-test.

5. ORIC CD73 inhibitors relieve intratumoral immune suppression

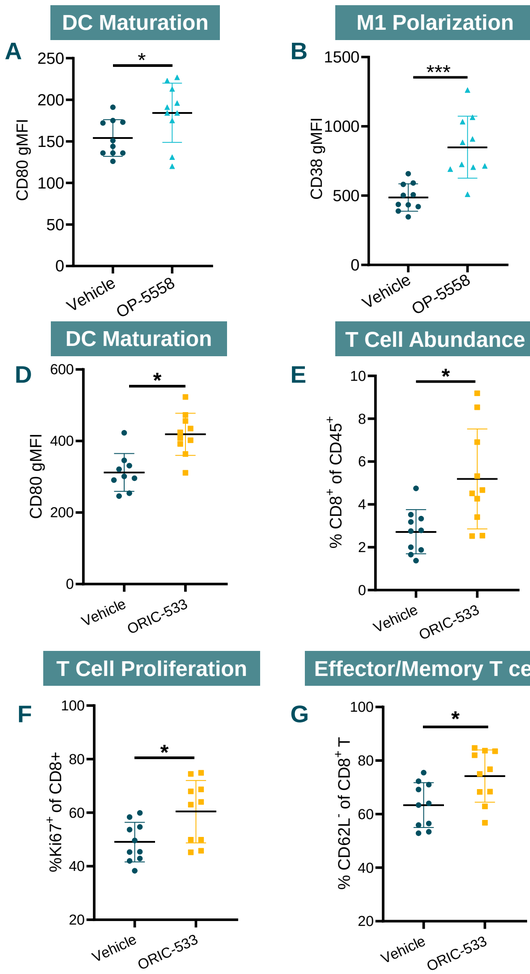


Figure 5. E.G7-OVA mice treated with OP-5558 or ORIC-533 demonstrate immune modulatory effects. **A.** OP-5558 administered through osmotic pump, at 15 mg/kg/d significantly increased intratumoral DC maturation and **B.** M1 macrophage polarization in terminal tumors. **C.** Immunohistochemical staining showed increased $CD8^+$ T cells in OP-5558 treated tumors. E.G7-OVA mice orally dosed with ORIC-533, PO QD for 19d at 150 mg/kg displayed significantly increased **D.** $CD11^-$ DC population, **E.** $CD8^+$ T cells, **F.** %Ki67 of $CD8^+$ T cells and **G.** effector and memory CD8+ T cells. Mean and SD are shown, *p<0.05; ***p<0.001; t-test.

CONCLUSIONS

ORIC's orally bioavailable small molecule CD73 inhibitors:

- potently suppress ADO production in vitro
- restore cytokine secretion of ADO-suppressed CD8+ T cells
- significantly reduce adenosine levels in tumors in vivo
- achieve significant tumor growth inhibition as single agents with oral delivery
- demonstrate in vivo immune modulation consistent with decreased immunosuppression

See also: AACR 2020 Poster #4317 CD73 inhibition with a novel orally bioavailable small molecule blocks adenosine production and rescues T cell activation

AACR Virtual Annual Meeting II, June 22-24, 2020, Poster #LB-115

Thank you to the ORIC CD73 team!