CD73 inhibition reverses immunosuppression and has potential as an immunomodulatory therapy in patients with multiple myeloma

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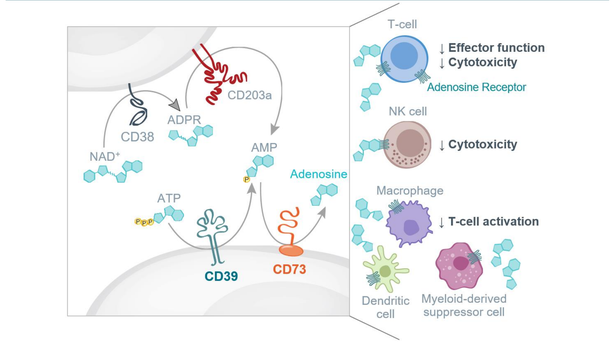
CD73 Mediates Immunosuppression and Therapeutic Resistance via Adenosine Production

Adenosine Pathway Components are Expressed in Multiple Myeloma



- Immunosuppressive adenosine generation from adenosine monophosphate (AMP) requires the activity of the cell surface ecto-5'-nucleotidase, CD73
- Relapsed/refractory (r/r) multiple myeloma (MM) is adenosine rich
  - Adenosine pathway components are highly expressed on MM cells and on many cell types within the MM niche
  - Adenosine levels in bone marrow are significantly higher in MM patients
  - High CD73 and adenosine are associated with poor prognosis and therapeutic resistance in multiple myeloma
- Plasmacytoid dendritic cells and multiple myeloma cells trigger tumor-promoting immunosuppression via CD73 pathway activation

Yang R *et al.*, J Immunother Cancer 2020; Horenstein A *et al.*, Mol Med 2016; Ray A *et al.*, Blood 2019; Ray A *et al.*, Blood 2021; Ray A *et al.*, Clinical Lymphoma Myeloma and Leukemia 2021; Ray *et al.*, Blood Cancer J 2022

1. Discovery of CD73 Inhibitors that Potently Suppress Adenosine Production

Biochemical and Cellular Potency of CD73 Inhibitors

Compound	Biochemical Potency IC_{50} (nM)	$CD8^+$ Potency EC_{50} (nM)
ORIC-533 clinical candidate	0.1	0.1
OP-5558 tool compound	0.1	0.05
AB680*	5.3	5

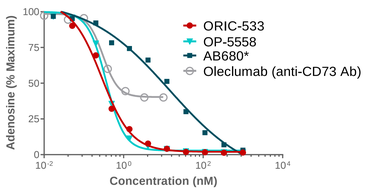


Figure 1. ORIC-533 and OP-5558 CD73 inhibitors have picomolar potency. OP-5558 and ORIC-533 effectively inhibit in vitro adenosine generation from AMP in human $CD8^+$ T-cells (table) and H1568 cells (left panel) at sub nanomolar concentrations as quantified by LC-MS/MS, with greater potency than clinical-stage small molecule and antibody inhibitors of CD73. *Bowman C.E. *et al.*, Biochemistry 2019, 58, 3331-3334.

2. ORIC Inhibitors Restore T-cell Function More Potently than Other Adenosine Pathway Inhibitors

Small Molecule CD73 Inhibitors | **Anti-CD73 Antibody**

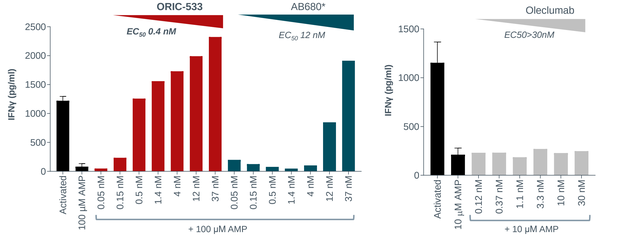


Figure 2. Human PBMC-derived $CD8^+$ T-cells were activated for 24hr with tetrameric anti-CD3/CD28/CD2 antibodies in serum-free media, labeled with CellTrace Violet and plated onto 96-well plates. Compounds and AMP were added at indicated concentrations and cells were incubated for 72-96hrs. Cytokines in cell supernatants were measured by MSD ELISA. Source: Sutimantanapi *et al.* AACR Poster 2021. iTeos from WO2020065036A1.

3. ORIC's Potent AMP-competitive Inhibitors are Active in a High AMP Environment

Cytokine production at 1mM AMP | **$CD8^+$ T-cell proliferation at 1mM AMP**

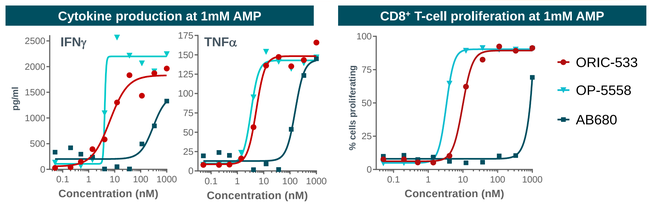


Figure 3. Human PBMC-derived $CD8^+$ T-cells were activated for 24hr with tetrameric anti-CD3/CD28/CD2 antibodies in serum-free media, as described in prior figure. Cytokines in cell supernatants were measured by MSD ELISA. Proliferating cells were quantified by flow cytometry. Sutimantanapi *et al.* AACR Poster 2021.

4. ORIC CD73 Inhibitor Triggers Single Agent Cytotoxicity in R/R MM Cells in Assay Utilizing Entire Bone Marrow Milieu

Autologous Ex Vivo MM-patient Bone Marrow Mononuclear Cell Cytotoxicity Assay

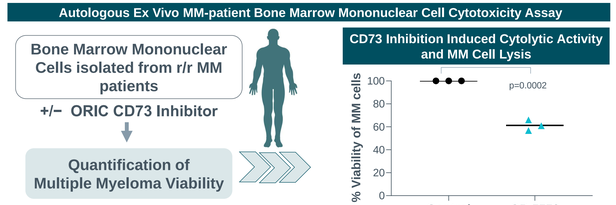


Figure 4. Relapsed/refractory MM-patient derived bone marrow mononuclear cells (BMNCs; at 1.25×10^6 cells/mL) were cultured in the presence of OP-5558 (0.5 µM) for 48 hours. MM cell viability was determined by flow cytometry using MM cell surface marker CD138 (n=3 r/r MM patient samples; mean ± SD; unpaired t-test).

5. ORIC Inhibitor Reversed Immunosuppression Resulting in T-cell Activation and Lysis of MM Cells from Relapsed/Refractory Patients

Autologous Ex Vivo T cell-mediated MM Cytotoxicity Assay

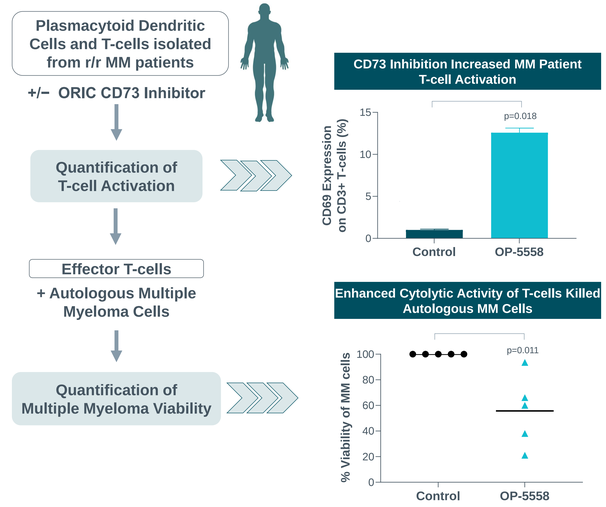


Figure 5: pDCs from relapsed/refractory (r/r) MM patients were cocultured with autologous T-cells at 1:10 (pDC:T) ratio in the presence or absence of CD73 inhibitor OP-5558 (0.5 µM) for 48 hrs. Viable $CD3^+$ T-cells were analyzed for CD69 activation (anti-CD3-FITC and anti-CD69-APC-Cy7 Abs) and quantified by FACS (n=3 r/r MM patient BM samples, mean ± SD; unpaired t-test). ***Top Panel:*** Change in the activation of $CD3^+$ T-cell populations in treated versus untreated. ***Bottom Panel:*** Scatter plot shows quantification of $CD138^+$ MM cells. The percent lysis was obtained after normalization with control data, and the graph is presented as percentage of viable cells in the presence and absence of OP-5558 (n=5 r/r MM patient samples; mean, unpaired t-test). MM samples were from relapsed or refractory patients who received at least 3 prior lines of therapy. All had prior proteasome inhibitor (PI) and lenalidomide, and other prior treatments included anti-CD38 monoclonal antibodies and CAR-T therapy.

6. ORIC-533 Reduces Adenosine Production in Bone Marrow from Relapsed/Refractory Patients

ORIC-533 Inhibits CD73 Activity in BM Serum from MM patients

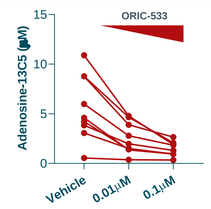
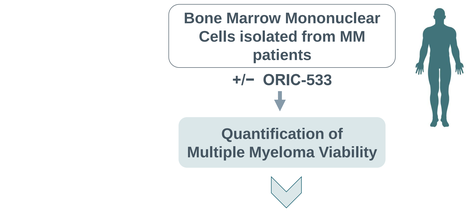


Figure 6. ORIC-533 inhibits CD73 activity and reduces adenosine generation from AMP in a dose-dependent manner. Serum from BM aspirates from relapsed refractory MM patients was incubated with AMP-13C5 for 15 minutes in presence or absence of ORIC-533. Two doses of ORIC-533 were assessed. Thereafter, the adenosine-13C5 was quantified by mass spectrometry.

7. Low Nanomolar ORIC-533 Triggers Single Agent Cytotoxicity in R/R MM Cells in Assay Utilizing Entire Bone Marrow Milieu

Autologous Ex Vivo MM-patient Bone Marrow Mononuclear Cell Cytotoxicity Assay



ORIC-533 Induced Cytolytic Activity Resulting in Autologous MM Cell Lysis

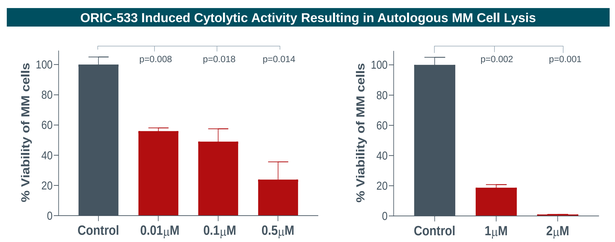


Figure 7. MM-patient derived bone marrow mononuclear cells (BMNCs; at 1.25×10^6 cells/mL) were cultured in the presence of ORIC-533 at indicated dose levels for 48-72 hrs. MM cell viability was determined by flow cytometry using MM cell surface marker CD138 (n=2 MM patient samples per dose range; mean ± SD; unpaired t-test). MM samples were from relapsed or refractory patients who received at least 3 prior lines of therapy or CAR-T therapy (left panel). MM samples were from patients responding to induction therapy or maintenance anti-CD38 monoclonal antibodies (right panel).

CONCLUSIONS

ORIC-533 exhibits potential best-in-class properties and is the first oral CD73 inhibitor to enter clinical development for multiple myeloma

- ORIC-533 is
  - a highly potent adenosine pathway inhibitor
  - superior in potency relative to comparator adenosine pathway inhibitors, even in high AMP environments
  - capable of activating plasmacytoid dendritic cells and increasing T-cell activation
  - effective at reducing adenosine generation in BM serum from relapsed/refractory multiple myeloma patients
  - able to trigger lysis of relapsed/refractory multiple myeloma cells as a single agent in autologous ex vivo assays

ORIC-533 Phase 1 Clinical Trial (NCT05227144) is Enrolling Patients with Multiple Myeloma