EOS100850 potently restores adenosine A_{2A} receptor-dependent suppression of T cell function in the adenosine rich tumor microenvironment

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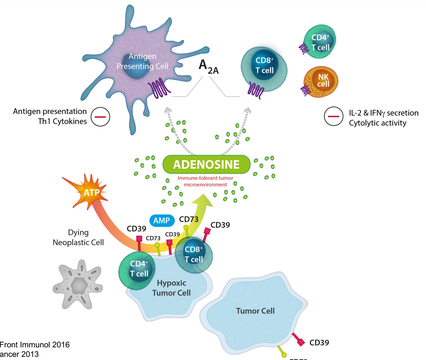
SUMMARY

- High levels of extracellular adenosine drive tumor immunosuppression
- Adenosine concentrations in tumors are at least 10-fold higher compared to normal tissue (see also poster #4147)
- Adenosine pathway markers CD39 and CD73 are strongly expressed in the tumor microenvironment on stromal cells as well as on tumor cells (see also poster #4446)
- A_{2A} receptor is the most prevalent adenosine receptor on immune cells
- Adenosine suppresses innate and adaptive immune reactions via signaling through A_{2A} receptors
- EOS100850 is specifically designed for immuno-oncology and has been characterized in various functional assays:
  - ✓ Enhances $CD8^+$ T cell priming
  - ✓ Restores antigen-specific $CD8^+$ T cell cytotoxicity suppressed by A_{2A} receptor activation
  - ✓ Rescues adenosine-mediated suppression of $CD4^+$ T-helper cell proliferation and function in a mixed lymphocyte reaction assay
  - ✓ Reverses A_{2A} receptor-dependent dysregulation of cytokine and chemokine production by innate and adaptive cells in whole blood

ADENOSINE-DRIVEN IMMUNOSUPPRESSION



Adapted from Ohta, Front Immunol 2016
Antonioli, Nat Rev Cancer 2013

HIGH EXTRACELLULAR ADENOSINE CONCENTRATION IN TUMORS

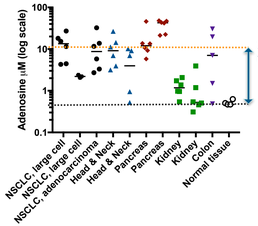


Fig. 1: Adenosine concentrations in patient-derived xenografts. Extracellular adenosine content was measured in 10 PDX from the indicated tumor histological types and normal tissue. Extracellular fluid from the PDX was obtained by microdialysis and adenosine was quantified by LC-MS. The mean adenosine concentration was 10.71 ± 1.70 µM (sem) (n = 5 to 6 tumors/PDX model; individual PDX and mean are shown).

STRONG CD39 AND CD73 EXPRESSION ON TILs AND TUMOR CELLS

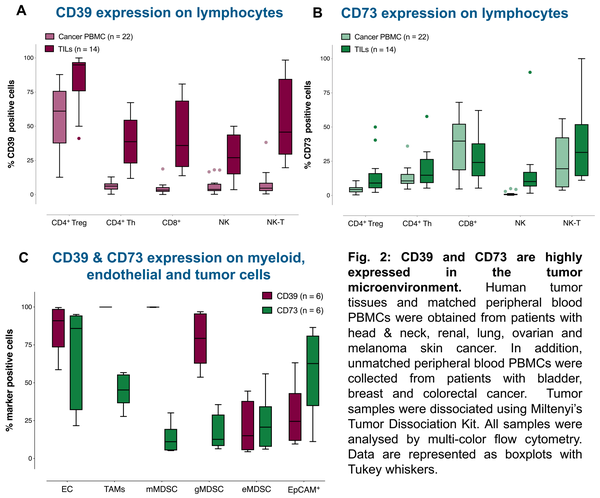


Fig. 2: CD39 and CD73 are highly expressed in the tumor microenvironment. Human tumor tissues and matched peripheral blood PBMCs were obtained from patients with head & neck, renal, lung, ovarian and melanoma skin cancer. In addition, unmatched peripheral blood PBMCs were collected from patients with bladder, breast and colorectal cancer. Tumor samples were dissociated using Miltenyi's Tumor Dissociation Kit. All samples were analysed by multi-color flow cytometry. Data are represented as boxplots with Tukey whiskers.

(A and B) CD73 and CD39 are expressed by peripheral blood lymphocytes of cancer patients, and their expression is moderately (CD73) to strongly (CD39) increased on tumor-infiltrating lymphocytes across multiple cancer types and across multiple lymphoid cell types. These data suggest that adenosine levels could be further increased in the TME compared to non-infiltrated PDX. (C) Tumor-infiltrating myeloid cells (MDSCs and TAMs) mostly express CD39, while EpCAM+ tumor cells mostly express CD73. Th: T-helper; NK-T: natural killer T cells; EC: endothelial cells; TAMs: tumor-associated macrophages; MDSC: myeloid derived suppressor cells; mMDSC: monocytic MDSCs; gMDSC: granylocytic MDSCs; eMDSC: early stage MDSCs.

A_{2A} RECEPTOR IS THE MAIN ADENOSINE RECEPTOR IN IMMUNE CELLS

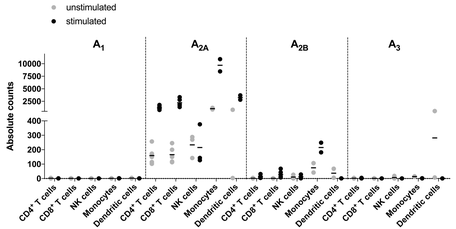


Fig. 3: Adenosine receptor mRNA quantitation by Nanostring nCounter. mRNA was prepared from immune cells isolated from human PBMCs. T cells and NK cells were purified by positive selection and stimulated for 3h with CD3/CD28 and IL-2 respectively. Monocytes were isolated by negative selection and stimulated with LPS for 3h. Immature dendritic cells were generated from positively selected monocytes, and matured during 24h in the presence of LPS.

EOS100850 ENHANCES $CD8^+$ T CELL PRIMING

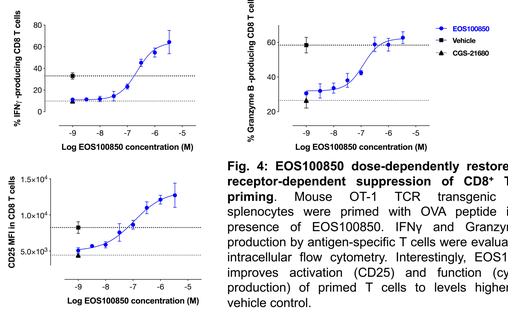


Fig. 4: EOS100850 dose-dependently restores A_{2A} receptor-dependent suppression of $CD8^+$ T cell priming. Mouse OT-1 TCR transgenic naive splenocytes were primed with OVA peptide in the presence of EOS100850. IFNγ and Granzyme B production by antigen-specific T cells were evaluated by intracellular flow cytometry. Interestingly, EOS100850 improves activation (CD25) and function (cytokine production) of primed T cells to levels higher than vehicle control.

EOS100850 RESCUES ANTIGEN-SPECIFIC $CD8^+$ T CELL CYTOTOXICITY

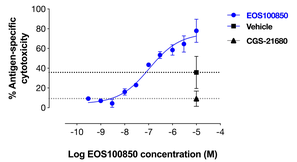


Fig. 5: EOS100850 dose-dependently stimulates mouse $CD8^+$ T cell cytotoxicity. Mouse OT-1 TCR transgenic splenocytes were primed with OVA peptide in the presence of EOS100850, and co-cultured with OVA-coated PanO2 tumor cells. A_{2A} receptor agonist CGS-21680 suppresses cytotoxicity. EOS100850 increases cytotoxicity to levels higher than vehicle. Improved function of cytotoxic $CD8^+$ T cells is likely due to enhanced T cell priming in the presence of EOS100850 (see panel above).

EOS100850 ENHANCES $CD4^+$ T-HELPER CELL PROLIFERATION AND FUNCTION IN A MLR ASSAY

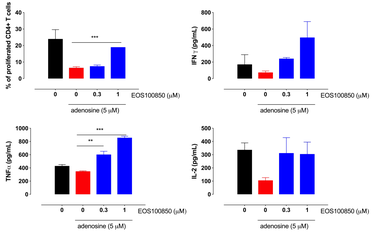


Fig. 6: EOS100850 dose-dependently reverses adenosine-mediated suppression of T-helper cell function in a mixed lymphocyte reaction assay. Monocyte-derived immature dendritic cells were co-cultured with allogeneic $CD4^+$ T cells in the presence of 5 µM adenosine and/or EOS100850. Mean ± SD is shown of a representative experiment of 2 independent experiments performed on 4 donor pairs. ** p<0.01, *** p<0.001; 1-way analysis of variance with Dunnett's multiple comparisons test.

Rac-EOS100850 REGULATES CYTOKINE AND CHEMOKINE PRODUCTION

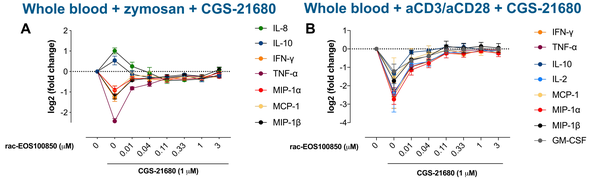


Fig. 7: rac-EOS100850 restores A_{2A} receptor-mediated modulation of (A) innate cell and (B) T cell-derived cytokines and chemokines in whole blood. Human whole blood was stimulated in the presence of 1 µM of A_{2A} agonist CGS-21680. n = 3 healthy donors. Zymosan is a stimulant of neutrophils and CD3/CD28 antibodies target T cells.

CONCLUSIONS

EOS100850 is a novel, best-in-class A_{2A} receptor antagonist designed for Immuno-Oncology

- Extracellular adenosine concentrations in tumors are strongly increased as compared to normal tissue. Therefore, A_{2A} receptor antagonists for the treatment of cancer need to be potent in high adenosine conditions.
- EOS100850 potently restores and enhances diverse immune cell functions in high adenosine conditions:
  - ✓ T cell priming, activation and proliferation
  - ✓ Antigen-specific cytotoxicity
  - ✓ Cytokine and chemokine secretion by innate and adaptive immune cells

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