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Intracellular adenosine drives profound lymphocyte suppression and can be reversed with EOS-984, a potent ENT1 antagonist

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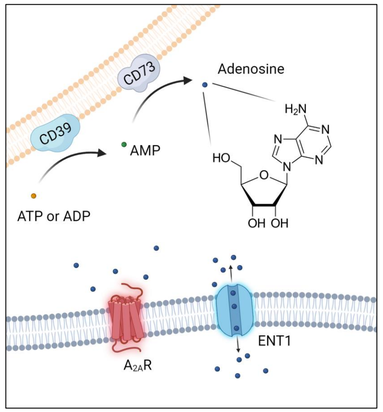
Introduction

The profound benefit of immune checkpoint blockade (ICB) for cancer therapy is restricted to limited subsets of patients with specific cancers. Novel targets and combination therapies are needed to improve treatment outcomes.

Factors contributing to ICB resistance include local accumulation of immunosuppressive metabolites such as the nucleoside adenosine. Adenosine promotes immune suppression through the A_{2A} receptor ($A_{2A}R$), expressed by tumor-infiltrating immune cells.

Pharmacological inhibition of adenosine generation (e.g. the enzymes CD39 and CD73) and signaling (e.g. $A_{2A}R$) are active areas of clinical investigation, however so far only limited clinical benefit has been reported.

Adenosine can enter cells through equilibrative nucleoside transporters such as ENT1. Whether and how intracellular adenosine influences anti-cancer immune responses is unknown.



Here, we show that high concentrations of extracellular adenosine found within the TME are taken up by T cells via ENT1 and impair their expansion and effector function. Furthermore, we have discovered and characterized EOS-984, a highly potent ENT1 antagonist blocking adenosine entry in T cells, which is currently under investigation in a Phase 1 clinical study in solid cancers.

Acknowledgements

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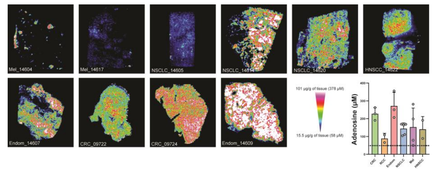
- Nucleoside transporter inhibition assays were performed at SOLVO Biotechnology (https://www.solvobiotech.com/)
- Human cell line xenograft experiments were performed at TransCure bioServices (https://transcurebioservices.com/)
- Introduction and conclusion schematics created in BioRender
- E.H. is an employee of and owns stock options/shares in iTeos Therapeutics, a company with ownership of EOS301984
- Study sponsored by iTeos Therapeutics

1. Very high adenosine concentrations are present within human tumors

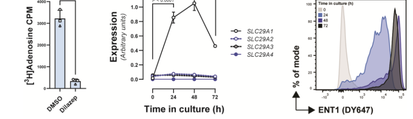
High tumor adenosine concentrations have important implications for cancer immunotherapy



Adenosine concentrations were assessed in tissue sections from 19 resected and frozen human tumors by quantitative mass spectrometry imaging (QMSI). Concentrations of adenosine exceeded 100 µM in the majority samples.

2. Adenosine enters activated human T cells via ENT1

ENT1 is the dominant transporter mediating adenosine uptake in T cells



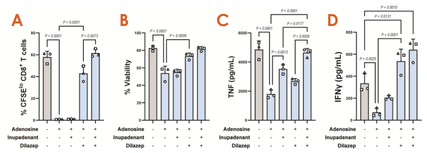
A. CD3/CD28-activated human T cells took up [^{3}H]adenosine which was blocked by the ENT1 inhibitor dilazep.

B. Human T cells upregulated *SLC29A1* (ENT1) transcription upon CD3/CD28 activation. Transcription of other ENT and CNT genes was not significantly affected by activation.

C. Human T cells upregulated ENT1 protein upon CD3/CD28 activation.

3. Adenosine uptake suppresses human T cell function and proliferation

ENT1 inhibition is an innovative approach to shield T cells from high tumor adenosine concentrations

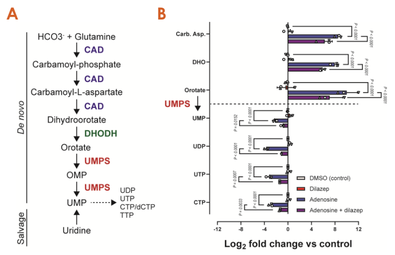


A. Adenosine (100 µM) suppressed the proliferation (CFSE dilution) of T cells following CD3/CD28 stimulation, which was restored mainly by the ENT1 inhibitor dilazep.

B-D. Summary data for T cell viability, TNF and IFNγ production, respectively, from experiments performed as in **A**. Symbols represent 3 donors.

4. Adenosine uptake suppresses *de novo* pyrimidine synthesis in human T cells

A novel suppressive mechanism of action of adenosine that is reversible via ENT1 inhibition

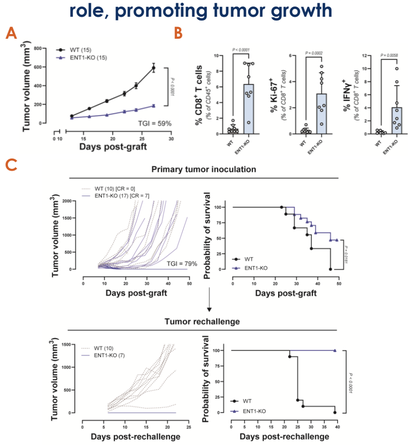


A. Pyrimidine *de novo* synthesis and salvage pathways.

B. T cells were CD3/CD28-activated for 24h in the presence of adenosine and/or the ENT1 inhibitor dilazep and pyrimidine metabolites were quantified by LC/MS. Metabolites upstream of UMPS were increased by adenosine (effect reduced by ENT1 inhibition), whilst metabolites downstream of UMPS were reduced by adenosine (levels restored by ENT1 inhibition).

5. Deletion of ENT1 leads to potent control of tumor growth *in vivo* with induction of effective immunological memory

ENT1 plays a key plays a key immunosuppressive role, promoting tumor growth



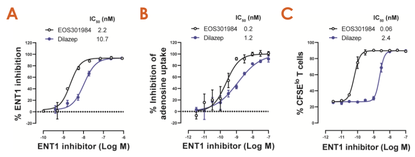
A. Growth curves of KPC s.c. tumors resistant to immuno-chemotherapy in WT and ENT1-KO mice, showing significantly reduced tumor growth in ENT1-KO mice.

B. Infiltration, proliferation and IFNγ production by CD8$^+$ TILs from KPC tumors were increased in ENT1-KO versus wt mice.

C. Growth curves of MC38 s.c. tumors in WT and ENT1-KO mice showing 7 complete tumor regressions (CR) in ENT1-KO mice which were then resistant to subsequent rechallenge.

6. EOS-984 is a potent ENT1 antagonist

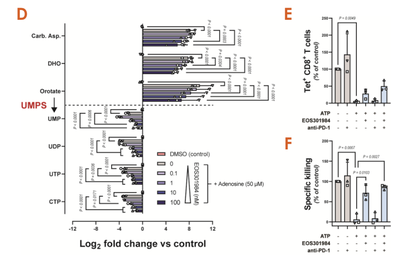
EOS-984 restores T cell activity suppressed by high adenosine concentrations with dramatically enhanced potency as compared with dilazep



A. ENT1 transport inhibition assay – EOS-984 inhibited ENT1-mediated uridine transport with nanomolar potency.

B. EOS-984 inhibited uptake of adenosine into CD3/CD28-activated human T cells with subnanomolar potency.

C. EOS-984 restored proliferation of activated T cells, suppressed by ATP (100uM; used as a source of adenosine), with sub nanomolar potency.

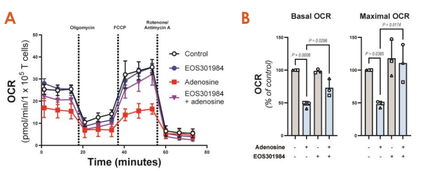


D. EOS-984 dose-dependently restored pyrimidine levels in human T cells activated in the presence of adenosine.

E-F. EOS-984 restored CMV pp65 peptide-specific T cell expansion in the presence of ATP (300 µM) as a source of adenosine, resulting in restoration of antigen-specific tumor cell killing, respectively. No restoration was observed with anti-PD-1 monotherapy.

7. EOS-984 prevents adenosine-mediated suppression of T cell mitochondrial function

EOS-984 supports metabolic function of T cells in high adenosine environments

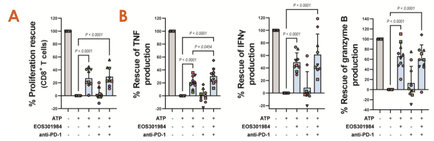


A. Seahorse Mito Stress Test analysis of T cell oxygen consumption rate (OCR) following 24h CD3/CD28 activation in the presence or absence of adenosine (50 µM) and EOS-984 (100 nM).

B. Summary of basal and maximal OCR of T cells from n=3 donors as in **A**.

8. EOS-984 protects tumor-infiltrating T cells from adenosine suppression

EOS-984 may enhance T cell expansion and function in high adenosine tumors

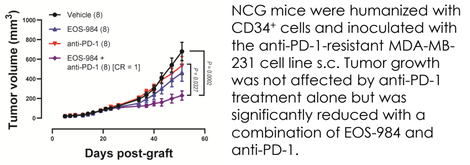


A. Human tumors were dissociated, and T cells were CFSE-labelled and stimulated with CD3/CD28 microbeads and IL-2 in the presence of ATP (500 µM) as a source of adenosine. ATP restricted T cell proliferation which was partially reversed by EOS-984 alone and in combination with anti-PD-1, whilst anti-PD-1 alone had almost no effect.

B. TNF, IFNγ and granzyme B levels were assessed in culture supernatants and were suppressed by ATP but restored in the presence of EOS-984.

9. EOS-984 synergizes with anti-PD-1 in restricting tumor growth in humanized mice

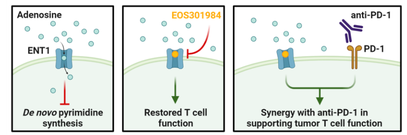
EOS-984 may extend the benefit of anti-PD-1 therapy to PD-1 resistant patients



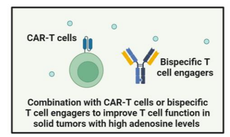
NCG mice were humanized with CD34$^+$ cells and inoculated with the anti-PD-1-resistant MDA-MB-231 cell line s.c. Tumor growth was not affected by anti-PD-1 treatment alone but was significantly reduced with a combination of EOS-984 and anti-PD-1.

Conclusions & perspectives

We have identified the uptake of adenosine through ENT1 on T cells as an important mechanism of immunosuppression within the adenosine-rich TME through inhibiting pyrimidine nucleotide synthesis. The potent ENT1 antagonist, EOS-984, relieves adenosine-mediated immunosuppression of tumor-infiltrating T cells and is currently being assessed in a Phase 1 clinical trial in advanced malignancies.



EOS-984 has potential as a combination partner beyond anti-PD-1, including with CAR-T cells and bispecific T cell engagers.



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