EOS100850, a non-brain penetrant highly selective A_{2A} receptor antagonist, uniquely maintains high potency within the adenosine rich tumor microenvironment

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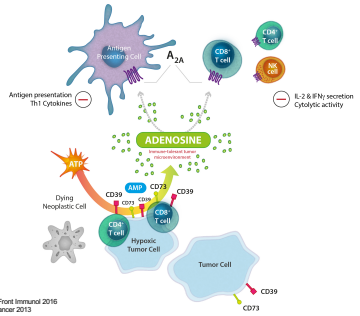
SUMMARY

- High levels of extracellular adenosine drive tumor immunosuppression through A_{2A} receptor signaling
- Adenosine concentrations in tumors are at least 10-fold higher compared to normal tissue (see posters #3278 and #4147)
- Clinical-stage competitor A_{2A} receptor antagonists dramatically lose potency in a high adenosine environment
- EOS100850 is specifically designed for immuno-oncology:
  - ✓ Highly potent in high intratumoral adenosine concentrations
  - ✓ Potency not affected by adenosine levels
  - ✓ Long residence time on A_{2A} receptor, resulting in prolonged PD effects *in vivo* (*in vivo* PD, see poster #4147)
  - ✓ Highly selective inhibitor of A_{2A} receptor
  - ✓ Non-brain penetrant, avoiding potential CNS side-effects at doses needed to inhibit tumoral A_{2A} receptors
  - ✓ Major metabolite is also a potent A_{2A} receptor antagonist, and the potency is not affected by adenosine concentrations

ADENOSINE-DRIVEN IMMUNOSUPPRESSION



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

EOS100850 IS HIGHLY POTENT IN THE ADENOSINE RICH TUMOR MICROENVIRONMENT

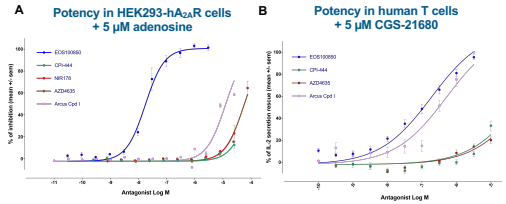


Fig. 1: EOS100850 is potent and outperforms clinical-stage competitors in conditions mimicking the tumor microenvironment. Dose-response antagonistic activity of A_{2A} receptor antagonists on human A_{2A} receptor in tumor-like conditions. (A) HEK293-hA2AR cells were stimulated with 5 µM of adenosine in the presence of 2% (w/v) human serum albumin (HSA). Generation of cAMP was used as a readout. Antagonist activity is expressed as a percentage of the inhibition of adenosine activity. (B) Primary CD3$^+$ T cells were stimulated with a combination of anti-CD3 and anti-CD28 antibodies and 5 µM CGS-21680, in the presence of 50% human serum (HS). IL-2 was quantified by alphaLISA after a 72-hour incubation. The normalized data, expressed as percentage of IL-2 secretion rescue over vehicle control, are shown. Arcus Cpd I based on example 1 from patent application no. WO/2018/136700.

EOS100850 Assay	IC_{50} in physiological environment	IC_{50} in tumor-like environment
Potency in HEK-hA$_{2A}$ (cAMP)	2.24 nM	21.8 nM
Potency in primary human T cells (cAMP)	1.87 nM	1.83 nM
Potency in CD8 T cells in whole blood (pCREB)	-	11.2 nM

Table 1. IC_{50} values of EOS100850 on human A_{2A} receptor in different assays mimicking physiological conditions and the tumor microenvironment. EOS100850 effectively inhibits A_{2A} receptor-mediated cAMP accumulation and pCREB formation, with IC_{50} values in the low nanomolar range. EOS100850 potency is hardly affected by challenging conditions mimicking the tumor microenviroment (i.e. high plasma protein and high A_{2A} receptor agonist conditions).

EOS100850 Assay	IC_{50} in physiological environment	IC_{50} in tumor-like environment
Potency in CHO-mA_{2A} (cAMP)	12.9 nM	66.8 nM
Potency in primary mouse T cells (cAMP)	6.1 nM	3.13 nM
Potency in mouse whole blood (pCREB)	-	5.25 nM

Table 2. IC_{50} values of EOS100850 on mouse A_{2A} receptor in different assays mimicking physiological conditions and the tumor microenvironment.

EOS100850 POTENCY IS NOT AFFECTED BY ADENOSINE LEVELS AND HAS A LONG RESIDENCE TIME ON A_{2A} RECEPTOR

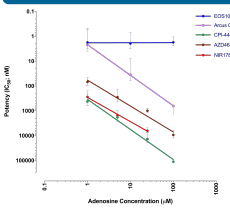


Fig. 2: EOS100850 outperforms competitors in normal and adenosine-rich environments, and its potency is not affected by adenosine levels. Human primary CD3 T cells were stimulated with different concentrations of adenosine (2% (w/v) HSA). cAMP was used as a readout. The potency of EOS100850 remains similar regardless of the adenosine concentration. As a result, EOS100850 is at least 100-10000 times more potent than competitors in the presence of 100 µM adenosine. Arcus Cpd I based on example 1 from patent application no. WO/2018/136700.

A_{2A}R antagonist	K_{on} (nM^{-1}•min^{-1})	K_{off} (min^{-1})	RT (min)	K_D (nM)
Rac-EOS100850	0.022 ± 0.010	0.006 ± 0.002	154 ± 48	0.30 ± 0.17
CPI-444	0.054 ± 0.003	0.045 ± 0.012	22 ± 6	0.83 ± 0.23
AZD4635	0.069 ± 0.029	0.181 ± 0.009	6 ± 1	2.6 ± 1.1

Table 3: Receptor binding kinetics for rac-EOS100850 and competitor antagonists on hA$_{2A}$ receptor. Association (K_{on}) and dissociation rate constants (K_{off}), residence times (RT), and (kinetic) K_D values are shown. The long residence time on A_{2A} receptor likely explains the 'insurmountable/non-competitive' profile of EOS100850, and results in a prolonged pharmacodynamic effect *in vivo* (see poster #4147).

EOS100850 IS A HIGHLY SELECTIVE A_{2A} RECEPTOR ANTAGONIST

Assay	EOS100850 IC_{50}	Selectivity vs. A_{2A}
A_1	0.192 µM	270-fold
A_{2B}	0.575 µM	1200-fold
A_3	> 30 µM	>40000-fold

Table 4. Activity of EOS100850 on human A_1, A_{2B}, and A_3 receptors.

EOS100850 IS NON-BRAIN PENETRANT

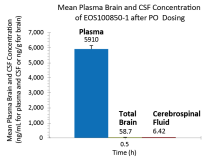


Fig. 3: EOS100850 concentrations in mice following oral administration. At T_{max}, as low as 1% of EOS100850 was detected in brain tissue compared to plasma. EOS100850 detected in the brain represented compound present in the residual blood in the collected brain tissue.

EOS100850's MAJOR METABOLITE IS A POTENT A_{2A} RECEPTOR ANTAGONIST

The major metabolite of EOS100850 (≈ 90%; EOS100612), identified both *in vitro* and *in vivo*, was synthesized and was found to be active on A_{2A} receptors. Characterization in several assays revealed similar characteristics as the parent compound, i.e. high potency in tumor-like conditions, not affected by adenosine concentrations.

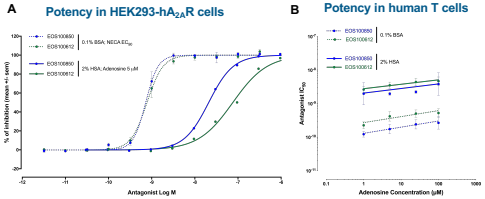
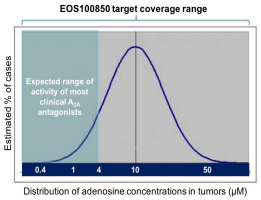


Fig. 4: EOS100612 is potent in normal and adenosine-rich environments, and its potency is not affected by adenosine concentrations. (A) Dose-response antagonistic activity of EOS100850 and EOS100612 on human A_{2A} receptor in normal and tumor-like conditions. Generation of cAMP was used as a readout. Antagonist activity is expressed as a percentage of the inhibition of agonist activity. (B) Human primary CD3 T cells were stimulated with different concentrations of adenosine. cAMP was used as a readout. The potency of EOS100850 and EOS100612 remain similar regardless of the adenosine concentration

EOS100612 Assay	IC_{50} in physiological environment	IC_{50} in tumor-like environment
Potency in HEK-hA$_{2A}$ (cAMP)	2.11 nM	73.2 nM
Potency in primary human T cells (cAMP)	2.40 nM	3.32 nM

Table 5: IC_{50} values of EOS100612, the principle metabolite of EOS100850, on human A_{2A} receptor

CONCLUSIONS



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

- Potent in high adenosine concentrations, a hallmark of the tumor microenvironment
- Potency is not affected by adenosine levels
- Long residence time on A_{2A} receptor
- No CNS penetrance and highly selective
- Major metabolite is highly potent A_{2A} receptor antagonist

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