EOS100850, An A_{2A} Receptor Antagonist with Prolonged Pharmacodynamic Activity, Mediates the Generation of Specific Durable Immune Responses in a Murine Breast Cancer Model

Pirson R. , Michaux A-C. , Jamart D. , Preillon J. , Frederix K. , Basilico P. , Martinoli C., Leroy X. , Driessens G. , Houthuys E. , Crosignani S. , Chappel S., Marillier R.

AACR 2019 #4147

SUMMARY

High extracellular adenosine in the tumor microenvironment which is the result of the activity of ectonucleotidases, such as CD39 and CD73, acts predominantly through the A_{2A} receptor (A_{2A}R):

- Supresses Th1 responses and cytotoxicity.
- Stimulates the activity of T_{regs}, MDSC and M2 macrophage differentiation.

iTEOS therefore developed an A_{2A}R inhibitor **EOS100850** which is:

- Non-brain penetrant.
- Selective inhibitor of A_{2A}R with sub-nanomolar Ki.
- Maintains its potency in high adenosine environment (see poster #3261).

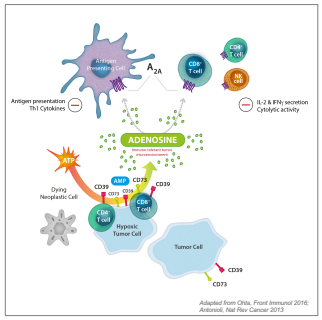
CREB phosphorylation (pCREB), a proximal readout for A_{2A} activation by adenosine or adenosine analogs (e.g. NECA), has been selected as a pharmacodynamic (PD) marker.

- EOS100850 demonstrated PD activity that extends well beyond its PK, which can be explained by a long residence time.

The adenosine pathway is active in the mouse syngeneic breast cancer tumor model, EMT-6. It has high CD39 and CD73 expression in the TME and adenosine levels are higher than concentrations found in non-tumor tissue. In this model EOS100850 administered in combination with anti-CTLA-4 mAb:

- Increased cytotoxic T cells and activated macrophages infiltration in TME.
- Suppressed tumor growth and increased the number of complete responders.
- Induced immunological memory.

ADENOSINE-DRIVEN IMMUNOSUPPRESSION



ITEOS A_{2A} RECEPTOR ANTAGONIST IS POTENT, SELECTIVE AND NON-BRAIN PENETRANT

Parameter	EOS100850	
	human	mouse
Potency in HEK-hA_{2A}/CHO-mA_{2A} (cAMP, IC_{50})	2.24 nM	12.9 nM
Potency in HEK-hA_{2A}/CHO-mA_{2A} high adenosine (cAMP, IC_{50})	21.8 nM	66.8 nM
Potency in human /mouse whole blood (pCREB, IC_{50})	11.2 nM	5.25 nM
Selectivity vs other adenosine receptors	> 270x vs hA_1 > 1200x vs hA_{2B} > 40000x vs hA_3	
CNS penetration	No	

ITEOS A_{2A} RECEPTOR ANTAGONIST DEMONSTRATES PD ACTIVITY THAT IS LONGER THAN PK

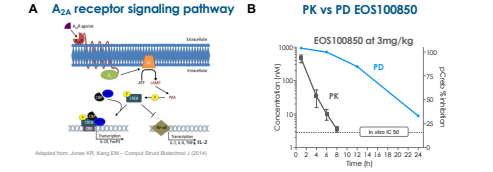


Fig. 2: EOS100850 inhibits A_{2A} receptor signaling in mouse whole blood PD assay. (A) CREB phosphorylation is a proximal indicator of A_{2A} activation. (B) EOS100850 concentration (PK) and inhibition of CREB phosphorylation (PD) were measured over time in mice treated with EOS100850 (p.o. 3mg/kg). For PK, EOS100850 concentration was measured in plasma by LC/MS. For PD, whole blood was incubated *ex vivo* with A_{2A} agonist (NECA), followed by pCREB detection by flow cytometry. The IC:50 on primary mouse T cells (cAMP assay) is also indicated, 3.13nM in high adenosine.

BREAST EMT-6 TUMOR MODEL EXPRESS CD39 AND CD73, CONTRIBUTING TO ELEVATED ADENOSINE IN TME

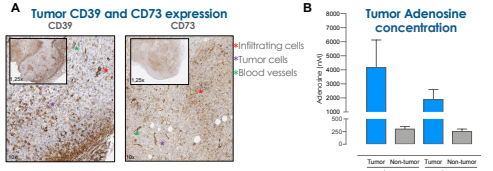


Fig. 3: Adenosinergic pathway is active in EMT6 model. EMT6 tumor cells were inoculated in syngeneic BALB/c. Preclinical microdialysis-based study was performed by Explicyte, to collect tumor and non-tumor fractions for adenosine quantification by LC-MS. Tumors were collected at the end of the sessions for IHC. **(A)** Illustrative IHC micrographs for CD39 and CD73 expression on tumor sections. **(B)** Adenosine quantification in the microdialysates at two different tumor size ranges. Data are represented as mean +/- SEM, adjusted for 20% recovery. N=5 mice/group.

ITEOS A_{2A} RECEPTOR ANTAGONIST EOS100850 INCREASES ANTI-TUMOR EFFICACY OF CHECKPOINT INHIBITORS

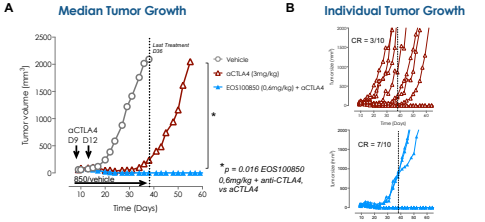


Fig. 4: EOS100850 enhances anti-tumor activity of aCTLA4. The efficacy of EOS100850 (0,6mg/kg QD) was evaluated in an established breast cancer syngeneic tumor model, EMT6, in combination with aCTLA4 (3mg/kg Q3Dx2, clone 9H10; BioXcell). The combination resulted in significant tumor growth inhibition. Median (A) and individual (B) tumor growth volume is shown; n=10 mice/group). The number of complete responders (CR) are also indicated. P value calculated using Linear mixed models statistical test.

EOS100850 IN COMBINATION WITH a-CTLA4 INDUCES A DURABLE, ANTIGEN-SPECIFIC MEMORY RESPONSE

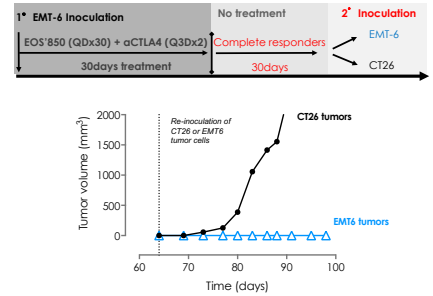


Fig. 5: EOS100850 in combination with aCTLA4 induces a robust immunological memory response. EOS100850 in combination with aCTLA4 treatment in EMT-6 challenged mice resulted in complete tumor regression in 7/10 mice (Fig. 4). To determine if a specific immune memory response was induced, treatment was suspended for 30 days, followed by re-inoculation of complete responders with EMT6 or irrelevant CT26 cells. The complete responders were protected against regrowth of EMT6 tumors but not irrelevant CT26 tumors (median tumor volume is shown; n=3-4 mice/group).

ITEOS A_{2A} RECEPTOR ANTAGONIST EOS100850 DRIVES A PRO-INFLAMMATORY RESPONSE

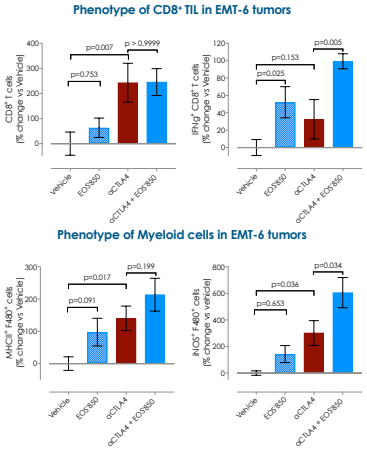


Fig. 6: EOS100850 in combination with aCTLA4 increases IFNg response, mediates activation and M1 macrophage differentiation. To investigate the effect of EOS100850 in the TME, EMT6 tumor bearing mice were treated for 7 days with Vehicle, EOS100850 (0.6mg/kg QD), aCTLA4 (1mg/kg Q3Dx2) or combination of EOS100850+aCTLA4. Tumors were removed and TIL were characterized by flowcytometry. Proportions are expressed as percent change compared to vehicle group. Data are Mean±SEM, n=10, P values calculated using One-way Anova statistical test.

CONCLUSIONS

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

- Potent in high adenosine concentrations, a hallmark of tumor microenvironment
- Prolonged in vivo PD activity
- In combination with check point inhibitor induces a durable anti-tumor response and antigen-specific memory
- Increasing functional activity of TILs and pro-inflammatory macrophages
- This profile of EOS100850 A_{2A} receptor antagonist supports its progression into clinical development.

iTeos Therapeutics, Gosselies, Belgium and Cambridge, MA, USA

Corresponding author: stefano.crosignani@iteostherapeutics.com