A novel adenosine A_{2A} receptor antagonist optimized for high potency in adenosine-rich tumor microenvironment boosts anti-tumor immunity

Erica Houthuys, Reece Marillier, Margreet Brouwer, Joao Marchante, Theo Deregnaucourt, Paola Basilico, Florence Nyawouame, Romain Pirson, Jakub Swiercz, Vanesa Bol, Gregory Driessens, Michel Detheux, Christophe Quéva, Stefano Crosignani, Bruno Gomes

AACR 2017
1683

ABSTRACT

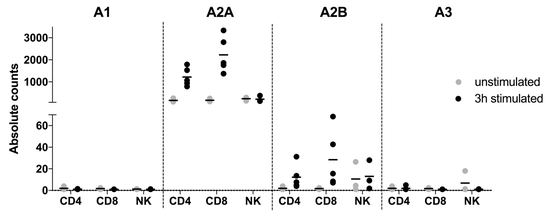
Extracellular adenosine in the tumor microenvironment is known to play a significant role in tumor immune evasion and promote tumor growth and metastasis (Ohta, 2016). We defined the receptor(s) required for mediating the effect of adenosine on immune cells within the tumor microenvionment and report the **characterization of a novel Immuno-Oncology-dedicated adenosine receptor 2A antagonist that functions in the high adenosine concentration found in tumors**.

We first explored the expression of the four adenosine receptors in primary human immune cells. **A_{2A} receptor was the main adenosine receptor** expressed by CD4 and CD8 T lymphocytes and monocytes, and the only one in mature monocyte-derived dendritic cells and NK cells. A_{2B} receptor was poorly detected in T cells and monocytes, while A_1 and A_3 receptors were never detected. Given these expression patterns, we further studied A_{2A} functions in primary human T lymphocytes and monocytes. Selective A_{2A} agonists strongly suppressed cytokine production by activated primary human T lymphocytes, thus highlighting that **A_{2A} is the main effector receptor for adenosine sensing in tumors**.

We developed tumor models with elevated extracellular adenosine levels by overexpression of CD73, the enzyme that converts AMP to adenosine. We showed that high adenosine levels correlated with strong tumoral expression of CD73. Interestingly, we showed that A_{2A} receptor antagonists designed for Parkinson's disease dramatically lost potency in a high adenosine environment. Our data indicated that a 30-fold dose increase may be required for full target inhibition within tumors.

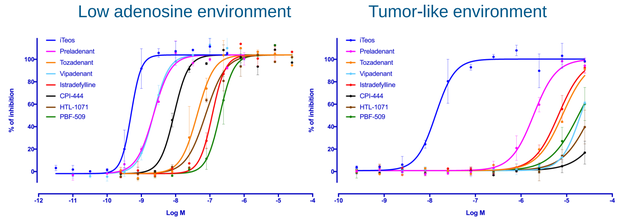
Therefore we developed a novel and potent A_{2A} blocker with sub-nanomolar Ki and IC_{50} in a cAMP assay and a more than 100-fold selectivity over other adenosine receptors. **Our lead compound maintained a high potency in an adenosine-rich environment and restored cytokine production even in the presence of high concentrations of A_{2A} agonists**. Furthermore, our compound was able to potently increase CD8 T cell cytotoxicity in a cytotoxicity assay with CD8 T cells as effectors and cancer cells as targets. These results suggest that **iTeos' new generation of A_{2A} receptor antagonist, designed to keep a high potency in the adenosine-rich tumor microenvironment, may offer a new therapeutic opportunity in Immuno-Oncology**.

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



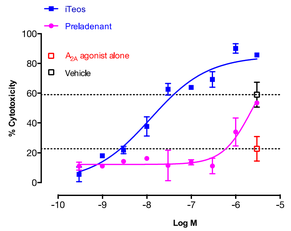
mRNA quantitation by Nanostring nCounter technology.

ITEOS A_{2A} ANTAGONIST IS HIGHLY POTENT IN ADENOSINE-RICH TUMOR MICROENVIRONMENT



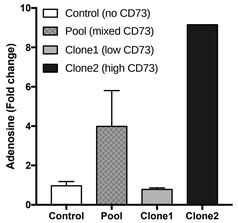
iTeos A_{2A} antagonist outperforms competitors in normal and adenosine-rich environment. Stimulation mimics normal (low adenosine) and tumor-like (adenosine-high, 2%HSA) environment, with cAMP used as a readout.

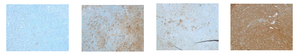
ITEOS A_{2A} ANTAGONIST INCREASES T CELL CYTOTOXICITY



iTeos compound abrogates A_{2A}-mediated inhibition of cytoxicity. OT1 cells, primed with Ova peptide in the presence of a high concentration of A_{2A} selective agonist and increasing concentrations of iTeos antagonist or Preladenant were then incubated with labeled Ova coated Panc02 cells as target cells.

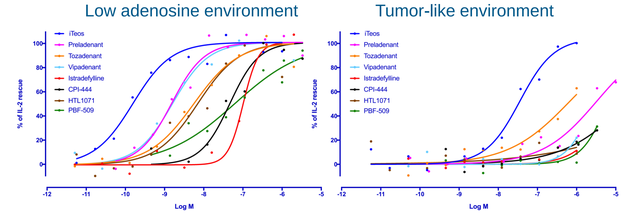
DEVELOPMENT OF ADENOSINE-RICH TUMOR MODELS





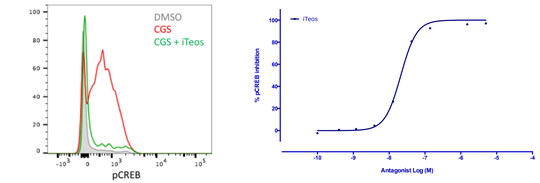
Adenosine levels in the tumor extracellular fluid correlate with CD73 expression. BALB/c mice were inoculated with a CT26 cell line that was transduced with an empty vector (Control) or with a vector expressing CD73 (Pool) or with clones expressing low and high levels of murine CD73. Adenosine was quantified in the tumor extracellular fluid.

ITEOS A_{2A} ANTAGONIST FULLY RESCUES HUMAN T CELL FUNCTIONS



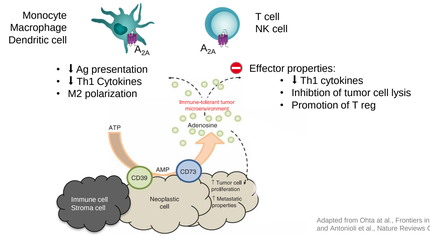
iTeos A_{2A} antagonist rescues T cell IL-2 production in high adenosine environment. Human primary $CD3^+$ T cells were stimulated in the presence of low or high (tumor-like) concentrations of A_{2A} agonist.

ITEOS A_{2A} ANTAGONIST FULLY BLOCKS A_{2A} SIGNALING IN IMMUNE CELLS



iTeos A_{2A} antagonist fully inhibits A_{2A} pathway activation in tumor-like conditions. Healthy donor peripheral blood lymphocytes were stimulated with a high concentration of A_{2A} selective agonist, with or without iTeos A_{2A} antagonist. Phosphorylation of CREB was analyzed by flow cytometry.

ADENOSINE-DRIVEN IMMUNOSUPPRESSION



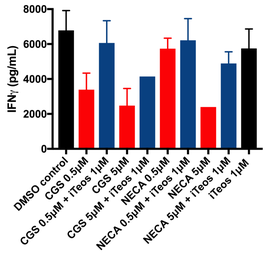
Adapted from Ohta at al., Frontiers in Immunology 2016 and Antonioli et al., Nature Reviews Cancer, 2013

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

Parameter	iTeos A_{2A} antagonist
Potency (cAMP, IC_{50})	< 1 nM
Potency in high adenosine (cAMP, IC_{50})	< 50 nM
Selectivity vs other adenosine receptors	> 100x vs hA_1 > 100x vs hA_3
CNS penetration	No

Given the higher level of adenosine in tumors when compared to the brain, much higher doses might be needed to achieve the desired effect on immune functions restoration for treating cancers. iTeos non brain-penetrant compound will avoid the CNS-related adverse effects that may appear in the dose escalation.

ITEOS A_{2A} ANTAGONIST INCREASES TH1 CYTOKINE PRODUCTION



iTeos A_{2A} antagonist restores A_{2A}-mediated suppression of T cell-derived IFNγ. Human peripheral blood mononuclear cells were cultured in 50% human serum and stimulated in the presence of A_{2A} agonists CGS21680 or NECA.

CONCLUSIONS

- **iTeos A_{2A} antagonist is a novel, best-in-class A_{2A} antagonist designed for Immuno-Oncology**
- iTeos A_{2A} antagonist is specifically designed to address the tumor microenvironment challenges
  - **Potent in high intratumoral adenosine concentration**
  - Limited CNS penetrance
- iTeos A_{2A} **antagonist fully rescues adenosine-driven T cell immunosuppression**

iTeos Therapeutics, Gosselies, Belgium

Corresponding author: bruno.gomes@iteostherapeutics.com

Wallonie
Service public de Wallonie
Avec le soutien de la DGO6
Département du Développement Technologique