Extensive characterization of the adenosine pathway in human solid tumors gives a hint on cancer indication selection for the A_{2A} receptor antagonist EOS100850

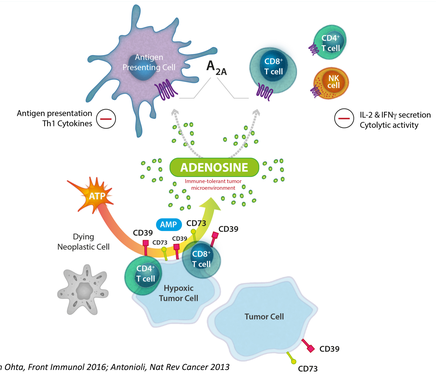
Wald N. , Martinoli C. , Mercier M. , Hermant A. , Nyawouame F. , Marchante J. , Moulin C., Bol V. , Crosignani S. , Bodo V.

AACR 2019 #4149

BACKGROUND

- High levels of extracellular adenosine promote immune suppression and is mainly generated by the sequential action of 2 ectonucleotidases: CD39 and CD73
- A_{2A} receptor is the most prevalent adenosine receptor of immune cells
- In the tumor microenvironment, adenosine concentrations are > 10-fold higher than in the normal counterpart, a major driver being hypoxia
- Adenosine signaling through A_{2A} receptor suppresses antitumor immunity
- iTeos A_{2A} receptor antagonist EOS100850 is designed for immuno-oncology:
  - ✓ Selective inhibitor of A_{2A} receptor
  - ✓ Potent regardless of the adenosine levels, in particular in high adenosine concentrations
  - ✓ Non-brain penetrant, avoiding potential CNS side-effects at doses needed to inhibit tumoral A_{2A} receptor
  - ✓ Rescues adenosine-driven T cell and innate cell immunosuppression
- iTeos A_{2A} receptor antagonist EOS100850 is in the clinic:
  - ✓ Phase 1/1B clinical study ongoing (FPFV in February 2019)
- Aim of this work is to support selection of cancer indications for EOS100850

ADENOSINE-DRIVEN IMMUNOSUPPRESSION



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

QUALIFIED ASSAYS TO MEASURE CD39, CD73 AND A_{2A} RECEPTOR EXPRESSION BY IHC IN CANCER TISSUES

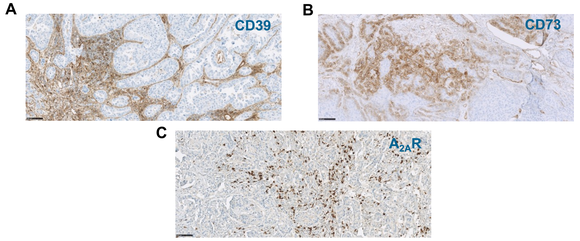


Fig. 1: Representative pictures of CD39, CD73 and A_{2A} receptor IHC staining on various human cancer tissues (lung, head and neck and breast cancer respectively).
Three IHC assays were developed to detect expression of CD39, CD73 and A_{2A} receptor in cancer tissues. Qualification of the staining included assessment of the specificity and sensitivity of the antibodies on cell lines with at least three different techniques among RT-qPCR, WB, IHC, FACS.
CD39 (A) and CD73 (B) ectonucleotidases were expressed by immune cells, endothelial cells and other stromal cells as well as by tumor cells, and their expression was increased in hypoxic regions.
A_{2A} receptor expression (C) was observed on different immune cell populations.

CD39, CD73 AND A_{2A}RECEPTOR ARE DIFFERENTIALLY EXPRESSED IN VARIOUS CANCER INDICATIONS

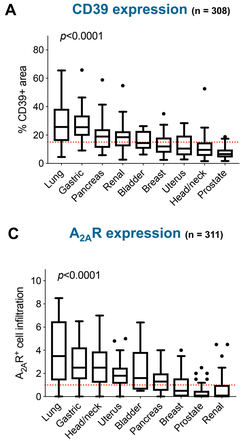


Fig. 2: CD39 , CD73 and A_{2A} receptor expression in human solid tumors. A. CD39 was quantified on 308 cancer donor samples. Lung and gastric tumors display the highest levels of CD39 expression, with 78% and 95% of samples, respectively, with CD39 above the median overall value (dotted red line).
B. CD73 expression was quantified on 318 cancer donor samples. Gastric, uterine and lung cancers display the highest levels of CD73 expression, with >70% of samples with CD73 above the median overall value (dotted red line).
C. A_{2A} receptor expression was quantified on 311 cancer donor samples. Lung, gastric and head and neck cancers display the highest levels of A_{2A} receptor with >80% positive samples (score >1, dotted red line).
Marker expression was analyzed by automated quantification (A and B, Visiopharm® software) or visual scoring (C) of IHC-stained tissue microarrays (TMA). Data are shown with Tukey plots. *p* values are from Kruskal-Wallis test.

EXPRESSION PROFILE OF ADENOSINE PATHWAY MARKERS AND IMMUNE INFILTRATE IN VARIOUS CANCERS

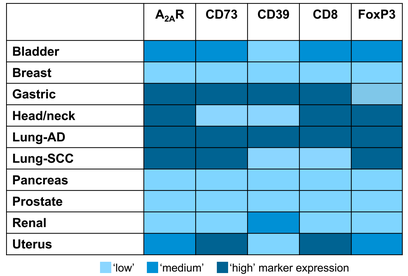


Fig. 3: Summary of adenosine pathway markers expression and immune infiltrate in human solid tumors
Markers of adenosine pathway, $CD8^+$ and $Foxp3^+$ T cells density were assessed in TMAs (n=13-53 samples per indication). Expression of these markers were classified in 3 categories (i.e. low, medium or high) according to their expression levels. AD: adenocarcinoma, SCC: squamous cell carcinoma

INFILTRATION OF A_{2A} RECEPTOR POSITIVE CELLS CORRELATES WITH IMMUNE INFILTRATE

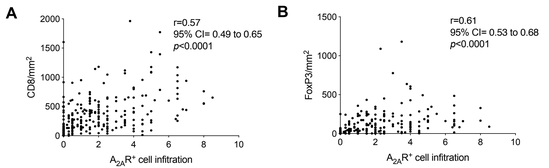


Fig. 4: A_{2A} receptor + cell infiltration correlates with $CD8^+$ T cells density (A) and $Foxp3^+$ T cells density (B). r coefficients were calculated with Spearman correlation. Each dot represents one sample.

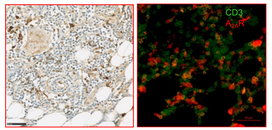


Fig 5. A_{2A} receptor is detected on T cells.
Chromogenic staining in cancer of A_{2A} receptor (left) and immunofluorescent double staining of A_{2A} receptor and CD3 (right) on breast cancer tissue. A_{2A}R is expressed by CD3+ T cells (yellow color).

HETEROGENEITY MARKER EXPRESSION IN DIFFERENT CANCER TYPES

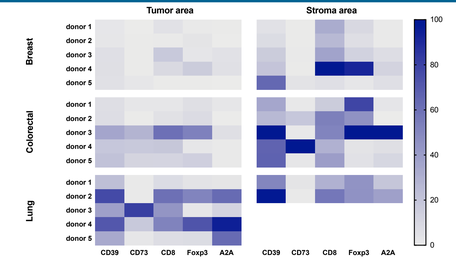


Fig. 6: Spatial distribution and heterogeneity of marker expression.
Markers of adenosine pathway and immune infiltrate were analyzed by IHC in five bulk tumor sections from donors with breast, colorectal and lung cancers. For each marker, expression levels were normalized to the highest value across the 15 samples.
CD39 expression as well as densities of A_{2A} receptor $^+$, $CD8^+$ and $Foxp3^+$ cells were all higher in the tumor surrounding stroma than in the tumor center in the three cancer types analyzed.

		CD8	Foxp3
TUMOR AREA	CD39	**r= 0.79 (95%CI: 0.44; 0.93) *p*= 0.0008**	**r= 0.60 (95%CI: 0.10; 0.85) *p*= 0.02**
	CD73	r= 0.48 (95%CI: -0.06; 0.80) *p*= 0.007	r= 0.14 (95%CI: -0.62; 0.42) *p*= 0.62
	A_{2A}R	**r= 0.74 (95%CI: 0.35; 0.91) *p*= 0.003**	**r= 0.56 (95%CI: 0.05; 0.84) *p*= 0.03**
STROMA AREA	CD39	r= 0.18 (95%CI: -0.45; 0.69) *p*= 0.57	r= 0.07 (95%CI: -0.53; 0.63) *p*= 0.84
	CD73	r= 0.26 (95%CI: -0.73; 0.39) *p*= 0.42	r= 0.29 (95%CI: -0.75; 0.35) *p*= 0.36
	A_{2A}R	r= 0.40 (95%CI: -0.25; 0.80) *p*= 0.20	r= 0.34 (95%CI: -0.31; 0.80) *p*= 0.29

Table 1: Correlation of markers expression in breast, colorectal and lung cancer sections
Correlations between immune (CD8 and Foxp3) and adenosine (CD39, CD73, A_{2A} receptor) markers were calculated in tumor and surrounding stroma areas.
CD39 and A_{2A}R significantly correlate in the tumor center but not in the stroma with CD8+ T cells and $Foxp3^+$ T cells densities, while CD73 expression does not correlate with $Foxp3^+$ T cells density neither in the tumor nor stroma areas.
Spearman correlation coefficients with 95% CI are shown.

CONCLUSIONS

- Three assays were successfully developed to measure CD39, CD73 and A_{2A}R by immunohistochemistry in human tumor tissues
- The assays were deployed to characterize adenosine pathway and its correlation with immune infiltration in nine different cancer indications.
- These data strongly support the relevance of targeting A_{2A}R and the adenosine pathway in immuno-oncology and pave the way to the identification of the cancer types that will benefit more from EOS100850.

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

Corresponding author: stefano.crosignani@iteostherapeutics.com