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A Novel Plasma Cell-Based Mechanism of Action of Adenosine Immunomodulation and $A_{2A}R$ Antagonism

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Introduction

High levels of extracellular adenosine are often found in the tumor microenvironment (TME).

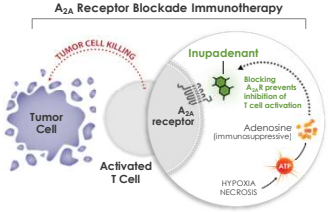
Extracellular adenosine promotes immune suppression mainly through the A2A receptor ($A_{2A}R$), expressed by tumor-infiltrating immune cells.

Inupadenant (formerly known as EOS100850) is an oral, non-brain penetrant, potent and highly **selective antagonist of $A_{2A}R$.**

In a Phase I/Ib clinical trial (NCT03873883), **inupadenant as monotherapy showed initial evidence of clinical benefit** in subjects with advanced solid tumors.

In the NCT03873883 study, **high baseline densities of $A_{2A}R$-expressing immune cells were associated with response or stable disease to inupadenant** (Buisseret et al, ASCO 2021 #2562).

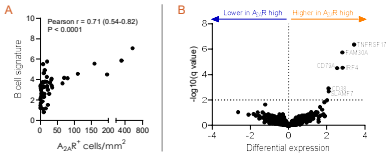
Current model for inupadenant-mediated restoration of anti-cancer immunity



Is there a role for other immune cells in inupadenant's mechanism of action?

Figure 1

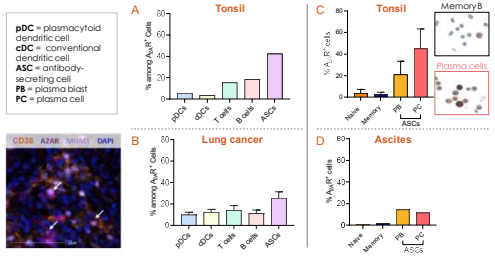
Intratumoral $A_{2A}R^+$ immune cells correlate with the expression of B cell- and antibody-secreting cell (ASC)-related genes



$A_{2A}R$ IHC and gene expression analyses were performed on 52 baseline biopsies from 27 subjects participating to NCT03873883. **A.** Correlation of infiltration of $A_{2A}R^+$ cells in the tumor area with a proprietary Nanostring B cell signature score. **The density of $A_{2A}R^+$ cells correlates with the expression of the B cell signature. B.** Differential expression of 780 genes according to level of infiltration of $A_{2A}R^+$ cells, and false discovery rate (FDR)-adjusted p values (q value, Benjamini and Yekutieli method). Names of B cell- and ASC-related genes are displayed. **All genes differentially expressed between patients with low vs high $A_{2A}R^+$ cell density are B cell- or ASC-related.**

Figure 2

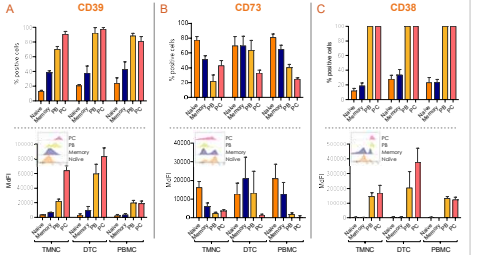
ASCs express $A_{2A}R$



ASCs are the major fraction of immune cells expressing $A_{2A}R$ in both tonsil and tumor, as assessed by two methods. Tonsil (n=1, **A**) and tumor samples (n=9, **B**) were stained by multiplex (m)IF and co-expression of $A_{2A}R$ was quantified. A representative mIF image of a lung tumor showing $A_{2A}R^+$ ASCs, denoted by arrows, is shown (**B**, left). Sorted B cell subsets from tonsillar mononuclear cells (TMNCs, n=3, **C**) and cancer ascites (n=1, **D**) were analyzed by immunocytochemistry (ICC) for $A_{2A}R$ expression. A representative ICC image of $A_{2A}R$ staining from a TMNC donor is shown (**C**, right).

Figure 3

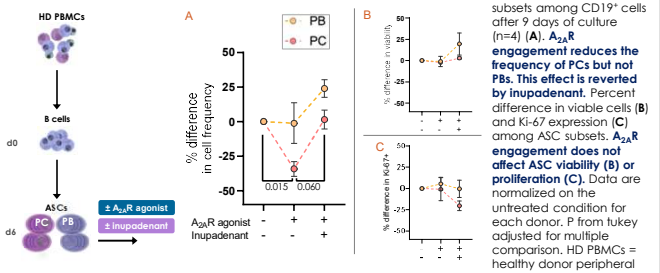
ASCs express high levels of AMP-generating ectoenzymes



Frequency (top) and median fluorescence intensity (MdFI; bottom) of CD39 (**A**), CD73 (**B**) and CD38 (**C**) on B cell subsets from healthy donor tonsillar mononuclear cells (TMNCs, n=5), dissociated tumor cells (DTCs, n=3) and cancer peripheral blood mononuclear cells (PBMCs, n=4). Inserts show representative histograms from one TMNC donor. MdFI is calculated on total cells. **ASCs are the major expressors of CD39 and CD38 in tumor and tonsil compared to other B cell subsets.**

Figure 4

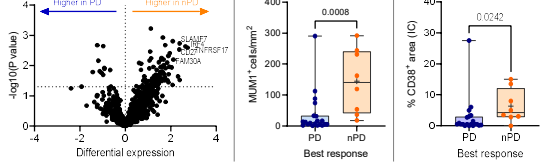
Inupadenant reverses $A_{2A}R$-mediated inhibition of plasma cell differentiation



Percent difference in ASC subsets among CD19⁺ cells after 9 days of culture (n=4) (**A**). **$A_{2A}R$ engagement reduces the frequency of PCs but not PBs. This effect is reverted by inupadenant.** Percent difference in viable cells (**B**) and Ki-67 expression (**C**) among ASC subsets. **$A_{2A}R$ engagement does not affect ASC viability (B) or proliferation (C).** Data are normalized on the untreated condition for each donor. P from tukey adjusted for multiple comparison. HD PBMCs = healthy donor peripheral blood mononuclear cells.

Figure 5

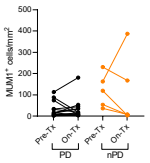
Baseline expression of B cell- and ASC-related markers is associated with best response on inupadenant



$A_{2A}R$ IHC and gene expression analyses were performed on baseline biopsies from 28 subjects participating to NCT03873883. PD = progressive disease; nPD= non-PD. (**A**) Differential gene expression according to best response to inupadenant. Names of B cell- and ASC-related genes are displayed. **Non-progressors express higher levels of several B cell- and ASC-related genes at baseline compared to progressors.** (**B**, **C**) Association between the infiltration of MUM1⁺ (**B**) and CD38⁺ (**C**) cells in the tumor area (assessed by IHC) and best response on inupadenant (PD, n=20; nPD, n=8). Each dot represents the mean of available biopsies. Box plot showing median and quartiles, with whiskers from min to max. + is the mean. P from Mann-Whitney. **Non-progressors display higher infiltration of MUM1 (IRF4)⁺ and CD38⁺ ASCs at baseline compared to progressors.**

Figure 6

Intratumoral ASCs decrease in non-progressors upon inupadenant treatment



Change in infiltration of MUM1⁺ cells in the tumor area (as assessed by IHC) in paired biopsies collected at baseline (Pre-Tx) and during treatment with inupadenant (On-Tx) from subjects participating to NCT03873883 study. Each dot represents the mean of available biopsies. PD, n=15: non-progressive disease, nPD, n=5. **Upon inupadenant treatment, the number of MUM1⁺ antibody-secreting cells (ASCs) is reduced in four out of five non-progressors, suggesting cell migration out of the tumor tissue.**

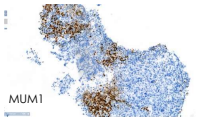
Figure 7

Case report: confirmed partial response to inupadenant in patient with highest ASC infiltration

Target lesion (TL)	Baseline	1st scan	2nd scan	3rd scan	4th scan
Subcarinal lymph node	15	14	11	9*	9*
Right hilar lymph node	15	15	10	6*	5*
% Reduction in TL		**3%**	**30%**	**50%**	**53%**

*TL = CR NTL= non-CR/non-PD at all visits

- Adenocarcinoma of unknown origin
- Prior progression on two lines of therapy
  - Prior Platinum doublet (4 months, BoR = SD)
  - Prior anti-PD-1 (7 months, BoR = PR)
- **Highest infiltration of antibody-secreting cells** observed to date (2438 MUM1⁺ cells/mm2) at baseline
- Inupadenant treatment ongoing (80mg BID)
- **Patient remains in partial response (confirmed in all subsequent scans Q8W)**



Conclusion & Interpretation

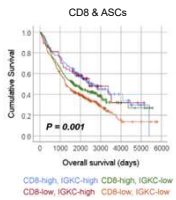
ASCs are a novel cellular target of inupadenant and $A_{2A}R$ antagonism.

The new **ASC-centric MoA may complement the well-known T cell-centric MoA.**

The **two MoAs could independently contribute** to inupadenant efficacy.

Our data are in line with the **emerging role of ASCs in the response to IO drug candidates.**

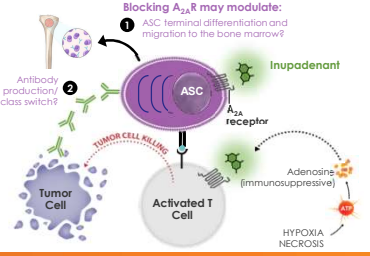
Why is this relevant? Key role of ASCs in cancer



- ASCs can infiltrate solid tumors.
- ASC infiltration is positively prognostic in several tumor types, regardless of T cell infiltration.
- ASC infiltration predicts response to aPD-(L)1.

https://doi.org/10.1038/s41568-022-00446-1
https://doi.org/10.1016/j.immuni.2022.02.001
https://doi.org/10.1016/j.ccell.2022.02.002
https://doi.org/10.1016/j.jtho.2018.12.022

Hypotheses under evaluation for inupadenant-mediated restoration of anti-cancer immunity via ASC modulation



NEXT STEP:

Determine which specific functions / subsets of ASCs are involved in the novel MoA:

- Terminal differentiation and migration to the bone marrow?
- Antibody production and class switch?
- Cytokine production?
- Antigen presentation?

Impact on clinical development

Evaluating association of ASC infiltration and outcome in ongoing randomized placebo-controlled Phase 2 trial in post-IO non-squamous NSCLC, in combination with platinum-doublet chemotherapy (secondary objective; NCT05403385).

Evaluating potential for patient enrichment/selection strategy based on ASC infiltration.

Methods

Immunohistochemistry (IHC) and Nanostring. Tumor biopsies were collected from patients treated with inupadenant monotherapy (NCT03873883) at baseline (Pre-Tx) and during treatment (day 21-27, On-Tx). FFPE blocks were analyzed by IHC. Gene expression analysis was performed using a customized Nanostring IO360 panel.

Multiplex immune fluorescence (mIF) analysis. FFPE blocks from adult tonsil and from lung tumor were analyzed by multiplexed immune fluorescence for the expression of PCK, $A_{2A}R$, MUM-1, CD19, CD3, CD11c, CD123, and CD38 in 39 regions of interest. Plasmacytoid (p)DCs were defined as CD123⁺, conventional (c)DCs as CD11c⁺, T cells as CD3⁺, B cells as CD19⁺MUM1⁻, ASCs as CD19⁺/⁻MUM1⁺CD123⁻CD11c⁻CD3⁻.

Flow cytometry analysis. Cancer peripheral blood mononuclear cells (PBMCs) from blood of cancer patients (lung, ovarian, kidney), fresh or frozen tonsillar mononuclear cells (TMNCs) from adult patients, and dissociated tumor cells (DTCs) from cancer patients (lung, ovarian) were stained with an antibody mix and analyzed by flow cytometry. The following gating strategy was used to identify different B cell subsets: naïve B cells (CD45⁺CD19⁺IgD⁺CD27⁻), memory B cells (CD45⁺CD19⁺IgD⁻CD27⁺ without CD38⁺⁺CD27⁺⁺ cells), plasma blasts (CD45⁺CD19⁺IgD⁻CD38⁺⁺CD27⁺⁺HLADR⁺) and plasma cells (CD45⁺CD19⁺IgD⁻CD38⁺⁺CD27⁺⁺HLADR⁻).

Immunocytochemistry (ICC) staining for $A_{2A}R$. Frozen TMNCs from healthy adult patients' tonsil and cancer ascites cells were stained with an antibody mix and naïve B cells, memory B cells, plasma blasts and plasma cells were sorted by FACS. $A_{2A}R$ expression was assessed by ICC on poly-L-lysin slides.

B cell culture. Freshly purified B cells isolated from healthy donor PBMCs were cultured for 6 days in 5%HS-xVivo medium containing human CD40-Ligand Multimer, anti-BCR, IL-21 and IL-2. Cells were incubated with or without CGS-21680 5 uM (an $A_{2A}R$ agonist) and with or without inupadenant 300 nM for 3 days. Cells were analyzed by flow cytometry.

Visualizations. Graphs show Mean + SEM, unless otherwise stated.

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