Identification of Adenosine Pathway Genes Associated with Response to Therapy with the Adenosine Receptor Antagonist CPI-444

Willingham S[1], Hotson A[1], Laport G[1], Kwei L[1], Fong L[2], Sznol M[3], Powderly J[4], Miller R[1]

[1]Corvus Pharmaceuticals, Burlingame CA, USA. [2]University of California, San Francisco CA, USA [3]Yale University School of Medicine, New Haven CT, USA [4]Carolina BioOncology Institute, Huntersville NC, USA

ABSTRACT

Background:
Extracellular adenosine in the tumor microenvironment generates an immunosuppressive niche that promotes tumor growth and metastasis by signaling through the A2A receptor (A2AR) on immune cells. CPI-444 is a selective A2AR antagonist that has demonstrated anti-tumor activity as a monotherapy and in combination with atezolizumab in an ongoing phase 1/1b trial in patients with advanced cancers. Here we analyzed gene expression profiles (GEPs) associated with A2AR agonism to characterize a "signature" of adenosine exposure in human immune cells and correlated this with GEPs in tumor biopsies from patients with renal cell cancer (RCC) treated with CPI-444.

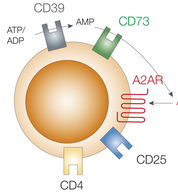
Methods:
Human PBMCs were stimulated with NECA (a stable analog of adenosine) or a specific agonist of A2AR (CGS21680). Purified RNA was analyzed using the NanoString PanCancer Immune Panel in conjunction with RNASeq. Select analytes were validated in culture supernatant by ELISA. RCC tumor biopsies collected from 64 patients treated with CPI-444 (100 mg BID) either as a single agent (n = 32) or in combination with atezolizumab (n = 32) were analyzed using NanoString.

Results:
In vitro A2AR stimulation resulted in dose-dependent increases in CXCR2 ligands (CXCL1,2,3,5,8) and key mediators of neutrophil/MDSC biology (CSF3, IL-23). Increases in monocyte/macrophage inflammatory mediators such as IL-1b and CCL2,3,7,8, 20 were also observed, as were increases in SERPINB2, S100A8, PTGS2, THBS1. Expression of CXCL10 and GZMB were decreased, consistent with a suppressed IFNg response. CPI-444 treatment inhibited these changes at the transcript and protein level. Preliminary biomarker analysis suggests CPI-444 anti-tumor activity in RCC was associated with increased expression of these adenosine responsive genes in pretreatment biopsies.

Conclusions:
A2AR agonists induce a specific gene signature dominated by immunosuppressive mediators of MDSC and monocyte/macrophage biology. Inhibition of these GEPs by CPI-444 are observed in vitro and in vivo in tumor biopsies from treated patients. These gene signatures may be used as biomarkers for patient selection.

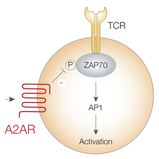
ADENOSINE INHIBITS ANTI-TUMOR IMMUNITY

Tumor & T_{REG} Cells



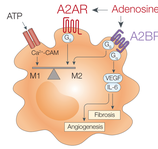
Increased FOXP3

$T_{EFFECTOR}$ Cell



Decreased IL-2 production
Increased CTLA-4 and PD-1 expression
Decreased proliferation

Macrophage

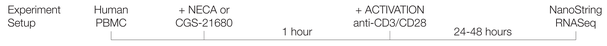


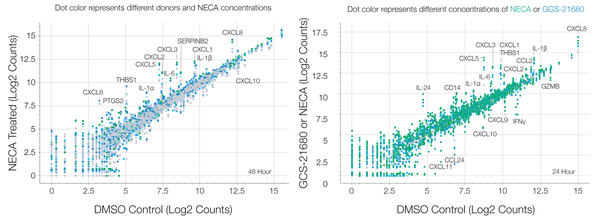
Promotes tumor supporting M2 phenotype
Increased angiogenesis

- Extracellular adenosine within the tumor microenvironment create an immunosuppressive niche that promotes tumor growth and metastasis.
- CPI-444 is a potent, selective inhibitor of the adenosine 2A receptor (A2AR).
- Extracellular adenosine has a short half life and it is not feasible to routinely measure in human tumors.
- This study aims to determine genes/proteins modulated by adenosine as surrogate signature to identify patients with adenosine rich tumors

A2AR AGONISTS INDUCE SPECIFIC GENE SIGNATURE

HUMAN PBMCs STIMULATED WITH NECA or A2AR SPECIFIC AGONIST CGS-21680





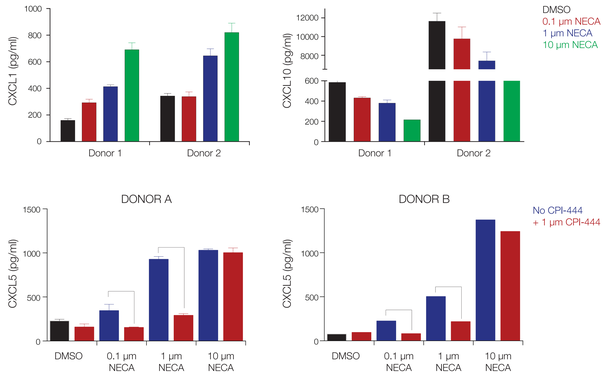
ADENOSINE SIGNATURE - NANOSTRING

	Adjusted p Value	Function	Receptor
IL23A	1.44E-04	Increases angiogenesis and reduces CD8+ T-cell infiltration	
SLC11A1	1.27E-03	Natural resistance-associated macrophage protein 1	
CXCL2	1.27E-03	MIP2a: macrophage inflammatory protein 2, alpha	CXCR2
CXCL7	1.27E-03	PPBP, Pro-Platelet Basic Protein	CXCR2
CXCL6	1.40E-03	GCP2: Granulocyte chemotactic protein 2	CXCR2
CXCL3	1.40E-03	Controls migration and adhesion of monocytes	CXCR2
IL-6	1.48E-03	Pro- and anti-inflammatory cytokine	
IL-1α	1.73E-03	Inflammation	
CXCL8	1.98E-03	IL-8. Nutrophil chemotactic factor	CXCR1/2
CXCL5	1.98E-03	Attracts and activates neutrophils	CXCR2
THBS1	2.28E-03	Multiple functions. Inhibits angiogenesis & immune regulation	
IL-1β	2.38E-03	Inflammation	
PTGS2	2.65E-03	COX-2. Elevated during inflammation and cancer	
IL-24	2.70E-03	Cell survival and proliferation. Activates STAT1/3	
CXCL1	3.92E-03	Neutrophil chemotractant	CXCR2
CD86	5.31E-03	B7-2: Costimulatory signal for T cell activation and survival	
CLEC5A	5.82E-03	Interacts with DAP-12 and may play a role in cell activation	
CD14	6.24E-03	Expressed by myeloid cells	

ADENOSINE SIGNATURE - RNAseq

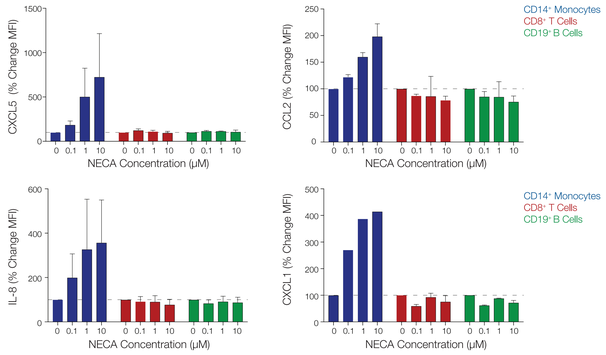
	Adjusted p Value	Function	Receptor
CXCL2	0.008	MIP2a: macrophage inflammatory protein 2, alpha	CXCR2
IL-23	0.008	Neutrophil attraction w/ IL-17, reduces CD8+ T cell infiltration	
CSF3	0.011	G-CSF, Master regulator of neutrophil development	
CXCL3	0.011	Controls migration and adhesion of monocytes	CXCR2
HAS1	0.014	Hyaluronan synthase 1, ECM component	
INHBA	0.016	Inhibin, beta a. Subunit of activin & inhibin	
CXCL5	0.019	Attracts and activates neutrophils.	CXCR2
PTGS2	0.019	COX-2. Elevated during inflammation and cancer	
PADI2	0.019	Protein-arginine deiminase type-2. Neurodegeneration	
CD93	0.023	C1qRp. Expressed on neutrophils. Clearance of apoptotic cells	
SCL7A7	0.024	Uptake of dibasic and neutral amino acids	
PID1	0.024	Phosphotyrosine Interaction Domain-Containing Protein 1	
ECEL1	0.024	Endothelin-converting enzyme-like 1	
CD300E	0.028	Expressed on myeloid cells. Interacts with TYRO	
ST6GALNAC2	0.028	Sialyltransferase 2	

CPI-444 NEUTRALIZES THE ADENOSINE SIGNATURE



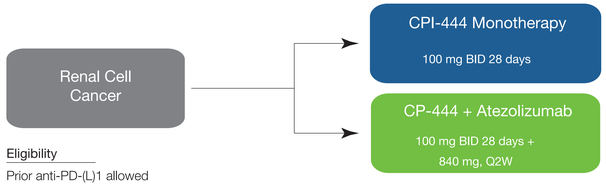
Purified human PBMCs from healthy donors were co-cultured with various concentrations of NECA and were stimulated with anti-human CD3 and CD28 antibodies for 1 hour. Culture supernatants were collected 48 hours later and chemokine expression levels were determined by ELISA. Adenosine signature related chemokine concentrations exhibited dose a dependent increase (CXCL1, top left panel) or decrease (CXCL10, top right panel). Addition of CPI-444 (1 μm) neutralizes the induction of CXCL5 by NECA as determined by ELISA (bottom panels).

ADENOSINE SIGNATURE PRODUCED BY CD14⁺ CELLS



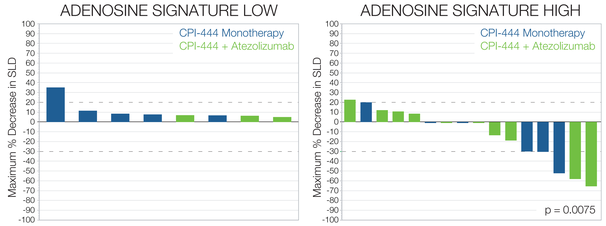
Purified human PBMCs from healthy donors were co-cultured with various concentrations of NECA and were stimulated with anti-human CD3 and CD28 antibodies. Cells were kept in culture for 2 days. Golgi block was added 4 hours prior to collecting cells for intracellular flow cytometry analysis. CD14⁺ monocytic cells exhibited elevated expression oftadenosine signature related cytokines and chemokines as NECA concentration increased. Lymphocytes including, CD8⁺ T cells and CD19⁺ B cells had minimal changes. These results indicate that the source of adenosine signature chemokines is likely to be of monocytic lineage

SIGNATURE CORRELATES WITH PATIENT RESPONSE



Eligibility

Prior anti-PD-(L)1 allowed
Progressive disease on prior therapy
No selection for PD-L1 expression



Adenosine Signature levels were determined in pre-treatment biopsy tissue. In brief, RNA was extracted from tumor tissue macrodissected from patient biopsy specimens and analyzed using the NanoString PanCancer Immune Panel. Cutoffs for determining adenosine signature high and low tumors were based on the mean of the Log2 NanoString counts of select adenosine signature genes for all subjects evaluated, including screen fails. The t-test for comparing the maximum percentage decrease in SLD between the adenosine positive and negative signatures is 2-sided p-value = 0.0075, using a 5% level two-sided test.

CONCLUSIONS

- A2AR agonists induce a specific gene signature in human immune cells. This "Adenosine Signature" is dominated by inflammatory myeloid cytokines and chemokines that signal through CXCR2.
- Adenosine induction of these genes dampens T cell immunity and shifts the balance away from T effector responses and toward myeloid suppressor functions.
- CPI-444 blocks the induction of the Adenosine Signature by A2AR agonists in vitro.
- Expression of the "Adenosine Signature" correlates with tumor regression in Corvus' ongoing Ph 1/1b trial with CPI-444 treatment in RCC. Patients with high expression of the Adenosine Signature were more likely to have tumor regression than those patients with low expression
- The Adenosine Signature may be used as a predictive biomarker that can be used to select patients most likely to respond to therapy with agents that antagonize adenosine production or signaling.
- Neutralization of the Adenosine Signature confirms the mechanism of action of CPI-444 as an A2AR antagonist.
- Further data along with an update on clinical results will be presented at the Society of Immunotherapy of Cancer (SITC) meeting in November 2018.