Co-inhibition of CD73 and A2AR Adenosine Signaling Improves Anti-tumor Immune Responses

Kris Sachsenmeier
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Overview

1. Introduction:
   - Adenosine in the Tumor Microenvironment

2. Targeting Adenosine
   - Genetics and Small / Large Molecules

3. Features of Targeted Combinations
A Key Role for Adenosine in Tumor Biology

Adenosine as “smog”…

NB: Adenosine impacts both myeloid and lymphoid compartments.
MEDI9447 Inhibits CD73 Enzyme Activity

IgG1, TM mutation for reduced Fc receptor engagement

Recombinant CD73

Cellular CD73

nM potency across three species.
Anti-CD73 Relieves AMP-mediated T Cell Suppression

Method: PBMCs → CD4+ → anti-CD3/28 w/ and w/o AMP + antibodies

Opportunities:
- Human system
- Patient PBMCs?

CONFIDENTIAL and Proprietary to Medimmune / Astrazeneca
Anti-CD73 Enhances Mixed Leukocyte Clustering

- No general (i.e. anti-CD3/28) stimulation….
Anti-CD73 Enhances a Two-Way Mixed Lymphocyte Reaction

- Timing and extent of PBMC clustering are increased.
- Dendritic cells are found at cluster centers.
• Targeting both CD73 and other modulators in a “mixed” setting shows synergy.
• Consistent with literature for various syngeneic models and signaling pathways
Non / Responders in Adenosine-targeted CT26 Tumors

- Beyond simple T cell targeting….to the entire microenvironment.

Hay et al., 2016
MEDI9447 + Anti-PD-1: Additive Activity at Optimal Doses

Tumor Growth Inhibition

Complete Response and Memory

- Established CT26 mouse CRC syngeneic model.
- Both antibodies used at the optimal (MBE / maximum biological effect) level.
- Combination better than either arm alone.
- “Cured” mice REMEMBER the tumor.

Hay et al., 2016
sCD73 Enzyme Activity in Cancer Patient Serum

- sCD73 activity was measured in sera from cancer patients or normal healthy donors.
- Enzyme activity is specifically inhibited by MEDI9447.
- Some cancer patients showed elevated soluble CD73 activity.
Adenosine as a Target

A2ARi
(ADO signaling)

Anti-CD73
(ADO generation)

After Leone et al., 2015
Double CD73/A2AR Knockouts Show Additive Effects

- Anti-PD1-resistant Melanoma
- Anti-PD-1 resistant Mammary Carcinoma
- Melanoma Metastasis

A2AR knockdown is doing something that CD73 knockdown is NOT doing.

Young et al., 2016

(Similar results with MCA-induced tumors.)
Mechanism(s) of Enhanced Tumor Growth Inhibition

- CD8 T cells are required.
- CD8 TILs also significantly increased.
- No changes in CD4, NK, myeloid infiltrates.

- Similar changes NOT observed in the tumor “margin”.
- Hypoxic response? CD73 is HIF1α responsive.

Young et al., 2016
Enhanced TGI From Pharmacologic Intervention

- Reflects genetic manipulation of A2AR and CD73
- Required: NK, CD8+ (Ab depletion), IFNγ and perforin (knockout mice).

A2ARi = SCH58261

Young et al., 2016
Anti-CD73 Fc Receptor Engagement in Human PBMC Activation

FcR engagement by Anti-CD73 reduces immune cell activation; myeloid and lymphoid.
Fc Receptor Engagement is Required for anti-CD73 Effects.

- FcγRIV but not FcγRIII is required; a small molecule CD73i was not additive with A2ARi

APCP = AMP analog; i.e. no FcR binding
Graphical Abstract

Young et al., 2016
Beyond Classical Checkpoint Inhibitors: Adenosine Inhibition

- Why should there be only one axis for tumor escape?
- Does adenosine signaling compensate when the PD-1 axis is blocked?

Modified from Lloyd et al., 2013
Thanks for your attention!
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Supplementary Information
Correlation Between Peripheral and Tumor cCD73 Enzyme Activity

A

Cellular CD73 Levels

% CD73+ Cells

Whole Blood Cells

Untreated  MED19447

Untreated  MED19447

B

Cellular CD73 Activity

RLU / cell

Tumor

% CD73+ Cells

p = 0.04

RLU / Cell

Untreated  MED19447

Untreated  MED19447

Whole Blood Cells (RLU/cell)

Tumor Cells (RLU/cell)

p = 0.04

Does periphery reflect the tumor?
Enhanced TGI From Pharmacologic Intervention

Combination effects seen in various models.

Young et al., 2016
Anti-CD73 + A2ARi Enhance TGI: Fc Receptor Engagement

Exact mechanistic role of Fc domain engagement under study.
Combination Effects Across Multiple Donor Pairs

Synergy generally reproducible.
What is Mass Spectrometry Imaging? DESI-MS?

How it works….

What can be done….

Metabolite (endogenous or drug)

Drug Biomarkers

Further analysis for required for identifications & quantitation

Relative intensity NOT absolute intensity
Preliminary DESI MS: Adenosine Levels in CT26 Tumors

Images chosen for demonstration; inter-sample variability being studied.

Richard Goodwin
Nicole Strittmatter
Carl Hay
Adenosine Levels in the Tumor Microenvironment

- ADO signaling is impacted differently across subsets in the “tumorhood”.
- CD73i ≠ A2ARi…..however, A1 / A3 can act oppositely from A2A/BR wrt cAMP.
What Spills From A Tumor Cell?

Method: Tritiated adenosine was traced after incorporation into PC3 prostate cancer cells.

- ATP, rather than adenosine, may be more likely from necrotic spill.
- CD73 is required for ATP → ADO transition.