Novel ‘elements’ of immune suppression within the tumor microenvironment

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NCAB meeting Sept 7, 2016
At the center of the galaxy of increasingly successful cancer immunotherapies

Cancer Vaccines

T cell:
Tumor cell

Checkpoint blockade
anti-PD-(L)1, anti-CTLA-4

CAR/TCR/TIL-based treatments
Understanding the tumor microenvironment during initiation and growth of tumor
Metastasis is the cause of >90% of all cancer deaths

- Successful metastasis requires evasion of immunity at the secondary site
- The lung is a common site of metastasis for many cancers
- Vascular architecture has historically explained cancer’s predisposition to disseminate to the lung

Hypothesis
Site-specific environmental factors – such as Oxygen – help establish immunologically permissive sites for metastasis
How do anti-tumor T cells ‘sense’ Oxygen, and does this affect their function?

T cells use prolyl hydroxylase domain (PHD) containing proteins

These dioxygenase (O₂) sensors containing non-heme–binding iron (Fe) that catalyzes the hydroxylation of proline residues
PHD proteins hydroxylate proline residues

Proline residue on PHD target protein
The PHD enzyme splits dioxygen into hydroxylated proline and succinate.
PHD enzymes degrade hypoxia inducible factor (HIF) – and possibly other proteins – in the presence of oxygen.
EGLN genes encoding PHD oxygen sensors are located at three different sites in human genome.

**PHD1 (EGLN2): 19q13.2**

**PHD2 (EGLN1): 1q42.1**

**PHD3 (EGLN3): 14q13.1**
Studying T cell-intrinsic oxygen sensing required a triple KO mouse

Does oxygen affect anti-tumor immunity?

Can oxygen sensing be manipulated to improve cancer immunotherapy?

D Clever, Cell, August 25, 2016
Oxygen Sensing by T Cells Establishes an Immunologically Tolerant Metastatic Niche

T-cell intrinsic PHD proteins suppress spontaneous pulmonary inflammation
T-cell intrinsic PHD proteins do not trigger spontaneous inflammation in the gut
CD4⁺ T cells lacking PHD proteins are prone to produce IFN-γ after stimulation.

D Clever, Cell, August 25, 2016
CD8$^+$ T cells lacking PHD proteins are prone to produce IFN-γ after stimulation.
**T cell-intrinsic expression of PHD proteins licenses tumor colonization in the lung but not SQ tissue**

- IV AND SubQ
- B16 melanoma
- WT or PHD-tKO

- WT
- PHD tKO

**WT**

**PHD tKO**

**Lung Tumors**

- WT
- PHD tKO

P < 0.01

NS
T cell-intrinsic expression of PHD proteins licenses tumor colonization in the lung but not SQ tissue.

IV AND SubQ

B16 melanoma

WT or PHD-tKO

Subcutaneous

Subcutaneous

Tumor area (mm²)

WT

PHD-tKO

Day Post Implantation

P < 0.01
PHD proteins suppress type I responses against innocuous house dust mite (HDM) Ag
Summary

1. T-cell intrinsic PHD proteins suppress spontaneous pulmonary inflammation

2. CD8\(^+\) and CD4\(^+\) T cells lacking PHD proteins are prone to produce IFN-\(\gamma\) after stimulation

3. T cell-intrinsic expression of PHD proteins licenses tumor colonization in the lung but not SQ tissue

4. PHD proteins suppress type I responses against innocuous house dust mite (HDM) Ag
The Problem

Normal homeostasis
Tumor colonization

Hyper-responsiveness to innocuous Ag
Tumor clearance
A Solution

Knockout or drug PHD proteins only in T cells specific for tumor antigens while leaving all other T cells intact
DMOG blocks the oxygen sensing PHD proteins

Dimethyloxalylglycine (DMOG)

proline + 2-oxoglutarate $\xrightarrow{\text{PHD2}}$ 4-hydroxyproline + succinate
Gene set enrichment analysis (GSEA) shows that DMOG/vehicle induces similar gene expression changes as PHD-tKO/WT.

**PhD-tKO/WT UP**

- **NES**: 4.18
- **P-value** < 0.0001
Inhibition of PHD proteins with DMOG before adoptive cell transfer immunotherapy

**Transferred Cell Function**

<table>
<thead>
<tr>
<th></th>
<th>Veh</th>
<th>DMOG</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ (pg/mL)</td>
<td>0</td>
<td>600</td>
</tr>
</tbody>
</table>

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TRP-1⁺ Splenocytes

Ex vivo expansion

+ DMOG

Subcutaneous OR Pulmonary Tumors
Inhibition of PHD proteins with DMOG before adoptive cell transfer immunotherapy

**Ex vivo expansion**

**TRP-1⁺ Splenocytes**

- **Veh**
- **+ DMOG**

**Transferred Cell Phenotype**

- **Foxp3**
- **T-bet**

**Subcutaneous OR Pulmonary Tumors**
Inhibition of PHD proteins with DMOG improves adoptive cell transfer immunotherapy

D Clever, Cell, 2016
Improved efficacy of DMOG-cultured cells for established subcutaneous tumors

D Clever, Cell, 2016
Foxp3⁺ iTreg fate specification of human CD4⁺ T cells cultured with DMOG

![Graph showing Foxp3+ iTreg fate specification](image)
Summary

1. DMOG blocks the oxygen sensing PHD proteins as evidenced by RNA seq and gene set enrichment analysis (GSEA)

2. Inhibition of PHD proteins with DMOG changes the function and phenotype of T cells . . .

3. . . . and improves adoptive cell transfer immunotherapy

4. Finally, similar maneuvers can be done with human CD4⁺ T cells
How do tumor immune suppressive mechanisms change with progressive growth?
Increased hypoxia accompanies progressive tumor growth

PHD function $[O_2]$ to cellular necrosis

- Hypoxia
- K$^+$ release
- Malignant solid tumor
- Normoxia

Restifo, In Preparation, 2016
The tumor microenvironment is characterized by a high tissue density of necrosis.

[Images showing tissue sections labeled 'Tumor', 'Necrosis', and 'Stroma' with labels 'Pt 4007-1' and 'Pt 4007-2' and magnification '4x'.]
Severe tumor necrosis is associated with a poor prognosis

Cumulative survival rate

None (n=15)
Moderate (n=69)
Severe (n=14)

Time from resection (days)

Komori et al. Anticancer Res. 2013
Necrosis releases intracellular ions into the extracellular space

Healthy Tissue

\[ [K^+]_i \approx 145 \quad [Na^+]_i \approx 5 \]

Interstitial (extracellular) space

\[ [K^+]_e \approx 5 \quad [Na^+]_e \approx 145 \]
Tumor interstitial fluid (TIF) has an elevated concentration of extracellular potassium ([K⁺])
Cell death correlates with levels of K$^+$ in the extracellular space

Background and Experimental Question

1. Human tumors persist and progress despite infiltration by tumor-specific effector T cells

2. Mouse and human tumors contain dense areas of cell necrosis

3. Cell necrosis leads to the release of an intracellular ion, potassium, into the extracellular space

4. Do elevated concentrations of extracellular potassium ([K⁺]) have any effect on T cell function?
Elevated [K⁺] acutely inhibits T cell effector function

Hyperkalemia augments checkpoint inhibition of T cells that may already be in place

Tumor Interstitial Fluid (TIF) contains ~ 40 mm of K⁺

1. Elevated [K⁺] produces profound suppression of human and mouse T cell TCR induced effector function

2. Hyperkalemia produces profound suppression of T cell receptor-induced transcripts including IL-2 and IFN-γ

3. Tumor associated hyperkalemia augments checkpoint inhibition of T cells that may already be in place
Naturally-occurring T cells express low levels of the potassium ion channel \textit{Kcna3} encoding \textit{Kv1.3}.

\textbf{R Eil, Nature (In Press), Fall, 2016}
Increased hypoxia accompanies progressive tumor growth

Restifo, In Preparation, 2016
Genetically engineering anti-tumor T cells to over-express the potassium ion channel *Kcna3*

*Kcna3* gene-engineered T cells make more IFN-γ *in vivo*
Anti-tumor T cells over-expressing Kcna3 have enhanced therapeutic efficacy

Overall summary


2. This local hyperkalemia produces profound suppression of human and mouse T cells.

3. T cells can be gene-engineered for resistance to hyperkalemia by over-expressing the $[K^+]$ ion transporter Kcna3.

4. Anti-tumor T cells over-expressing Kcna3 have enhanced therapeutic efficacy.
Tumor-induced immunosuppression is complicated.

Hargadon, Front. Immunol., 2013
## Composition of a human being

<table>
<thead>
<tr>
<th>Element</th>
<th>Symbol</th>
<th>Percentage in Body</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen</td>
<td>O</td>
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<tr>
<td>Carbon</td>
<td>C</td>
<td>18.5</td>
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<tr>
<td>Hydrogen</td>
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<tr>
<td>Nitrogen</td>
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<tr>
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<tr>
<td>Potassium</td>
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<td>Sulfur</td>
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<tr>
<td>Sodium</td>
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<tr>
<td>Chlorine</td>
<td>Cl</td>
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<tr>
<td>Magnesium</td>
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</table>

Trace elements include boron (B), chromium (Cr), cobalt (Co), copper (Cu), fluorine (F), iodine (I), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), silicon (Si), tin (Sn), vanadium (V), and zinc (Zn), less than 1.0
What is the immunology of the elements and how can it be used to destroy cancer?
Acknowledgements

Restifo Lab:
Past and present

Robert Eil
David Clever
Madhu Sukumar
Douglas C Palmer
Suman Vodnala
Shashank Patel
Christopher Klebanoff
Raul Vizcardo
Zhiya Yu
Ping-Hsien Lee
Devikala Gurusamy
Christine Kariya
Rigel Kishto
Anthony Leonardi
Marta Bosch-Marce
Arash Eidizadeh
Amanda Henning

Luca Gattinoni
Yun Ji
Rahul Roychoudhuri
Enzo Bronte
Willem Overwijk
Christian Hinrichs
Nick Acquavella
Joe Crompton
Nick Klemen
Tori Yamamoto
Naritaka Tamaoki
Rafiqul Islam

Collaborators:

John O’Shea
Jon Yewdell
Yasmine Belkaid
Ananda Goldrath
Rafi Ahmed
Carl June
Francis Collins

Rosenberg Lab:

Eric Tran
Alena Gross

Clinical Team:

James Yang
Udai Kammula
Rick Sherry
Stephanie Goff
Paul Robbins
Steve Feldman
Robert Somerville
Steve Rosenberg

David Stroncek
Franco Marincola
Ena Wang
Increased hypoxia accompanies progressive tumor growth

- PHD function $[O_2]$
  - ↓ Function of Kv1.3
- Hypoxia
  - Malignant solid tumor
  - K$^+$ release
- Normoxia
  - Cellular necrosis
  - ↑ Release of K$^+$

Restifo, In Preparation, 2016