Presenter Disclosure Information

James Finke
The following relationships exist related to this presentation:

Pfizer- Research Grant
Regulatory Immune Cells

James H Finke, PhD
Cleveland Clinic
Regulatory T cells in Tumor Immunity

- **Maintain Immune Tolerance**
  - Inhibit autoreactive T cells
  - Transfer of CD25+ depleted CD4+ T cells into nude mice results in autoimmune disease.
  - In humans, mutations of FoxP3 gene impairs development/function of Tregs – autoimmunity.
  - Ectopic expression of FoxP3 in normal T cells converts them to Treg.

- **Suppress Anti-tumor Immunity**
  - Tumor rejection in mouse models correlated inversely with suppressive activity of CD4+ T cells.
  - Depletion of Treg in vivo (CD4+CD25+Foxp3+) reduces tumor growth in mouse models.
**Inducible Tregs (Foxp3⁺)**
(Differentiated outside of the Thymus)

Origin: Naïve T cells CD4⁺CD25⁻
Activated effector/memory T cell (CD4⁺CD25⁻)

Induction signals:  
- TCR stimulation  
- IL-2, TGFβ  
- DC/MDSC/Tumor
Treg Induction with RCC cell line (SK-RC-26b), MDSC and anti-CD3/CD28 Stimulation

% Tregs in total CD4+ Population

- **No Stim**
- Stim (anti-CD3 Ab/anti-CD28 Ab)
- MDSC 1:1 Stim
- MDSC 1:5 Stim
- MDSC 1:10 Stim

**Legend:**
- Blue: Media (n=6)
- Red: RC26b Transwells (n=5)

**Significance Levels:**
- p=0.05
- p=0.03
- p=0.0001
- p=0.0004
- p=0.006
- p=0.001
The CD4+CD25+ High Cells Induced by CoCulture of CD4+CD25- Cells with Tumor Cells are Functionally Suppressive

Additive Of Feeders Only, CD25- Only and CD25+ High Only

Values are % suppression based on the additive ³H-thymidine counts

³H-Thymidine Counts


Feeder Only  84.8%  88.8%  54.5%
Treg Numbers in Cancer Patients

- Increased number of tumor infiltrating FoxP3+ cells (by immunostaining) associated with poor prognosis (Ovarian, Hepatocellular, cervical and Head and Neck Squamous Cell Carcinomas)

- No association in Renal Cell Carcinoma

- Frequently increased in the blood of some cancer patients (RCC etc. – no correlation with poor prognosis)

- Suppressive *in vitro*
Treg Cell Analysis

Increased Treg in Peripheral Blood of RCC Patients

Increased % of Treg Cells in the CD4+ Population

\[\text{Mean} \pm \text{SEM}\]

Normals
\[n=20\]
mRCC
\[n=34\]

\[p=0.002\]
Assay for Treg Suppressor Function Using RCC Patient PBMC

Sorted Tregs from RCC Patient & Normal : Proliferation Assay

- CD4+CD25- vs CD4+CD25+high
- Additive Allo Stimulation (1:1) (5:1) (10:1)

CPM

CD4+CD25:CD4+CD25+high

Allo Stimulation

- Patient
- Normal
Mechanisms of Treg Function

- Cytokines
  - TGFβ
  - IL-10

- Galectin 1 (β-galactoside binding protein)
  - blocking reduces function

- Granzyme B
  - apoptosis

- Inhibition of DC function
Other Regulatory T cells

**Tr1/Tr3**
- Antigen Induced
- Tr1 secrete IL-10, Tr3 secrete TGFβ
- No Specific Markers
- FoxP3 not constitutively expressed
- CD4^+CD25^{int} T cells secreting IL-10, not IFN, detected in some human tumors (Gastric Cancer, Renal Cell Carcinoma).

**CD8^+ Treg Cells**
Immunosuppressive populations include:
- CD8^+CD25^{+}FoxP3^{+}
- CD8^+IL10^{+}

**NKT regulatory cells**
Targeting Tregs

- **Targeting CD25 Receptor**
  - Ontak (Denileukin Diftitox, IL-2/Diphtheria toxin fusion protein) +/- vaccine
  - Recombinant anti-CD25 immunization
  - Immunized IgG1 monoclonal antibody to CD25 +/- vaccine
    - Varying degrees of decreasing Tregs
    - Increase Th1 response

- **Cyclophosphamide**
  - Augments cellular and humoral regions
  - Deplete Treg and boost efficacy in mouse models

- **CpG**
  - Lowers Foxp3+ T cells in lymph nodes of Melanoma patients

- **Block Treg Function**
  - Stat 3 decreases function (TKI Sunitinib)
  - Ox40

- **Block Treg Differentiation**
- **Block Trafficking**
Sunitinib Treatment Reduces Treg in RCC Patients Peripheral Blood.

- Pretreatment: n=48
- Cycle 1 Day 28: n=48
- Cycle 2 Day 28: n=29
- Cycle 4 Day 28: n=18

p-values:
- Normals vs Pretreatment: p < 0.0001
- Pretreatment vs Cycle 1 Day 28: p = 0.90
- Cycle 1 Day 28 vs Cycle 2 Day 28: p = 0.19
- Cycle 2 Day 28 vs Cycle 4 Day 28: p = 0.02
Reduction in CD4+Foxp3+ Treg cells in tumor and draining lymph nodes after treatment (B16) with sunitinib, vaccine or both.
Myeloid-derived Suppressor Cells

- Immunosuppressive myeloid cells
- Normally present in very small amounts but systemically accumulate under pathologic conditions – tumor-bearing
- Accumulation associated with:
  - VEGF, SCF, GM-CSF, G-CSF, S100A9, and M-CSF

MDSC depletion in murine tumor models:
- Inhibits/slow tumor formation
- Allows for immune-mediated tumor destruction
- Reduces tumor metastasis
- Adoptive transfer of MDSC into tumor bearing mice promotes tumor growth and inhibits T cell activation.
Bone Marrow

GM-CSF
M-CSF
SCF
Flt3
IL-3

Immature Myeloid Cells

Migrate to peripheral organs

Differentiate

DCs
Macrophages
Granulocytes

Normal Environment

Tumor Environment

Immature Myeloid Cells

• Accumulate
• Prevent Differentiation
• Induce T cell suppression
**MDSC Expansion**

**VEGF**
- Increased levels in cancer
- Promotes tumor vasculature
- Induces defective differentiation
- VEGFR1+ cells detected

**Prostaglandin**
- Overexpression of COX2
  - PGE2 induces MDSC

**GM-CSF**
- Produced by human and mouse tumors
- Promotes differentiation of myeloid precursors
- Bone Marrow cells + GM-CSF become immunosuppressive cells
- Not sufficient alone
- Excessive amounts may be responsible for induction of MDSC
  - Administration of GM-CSF-based vaccine
  - Increased MDSC numbers.

**Stem Cell Factor, IL-6, M-CSF**
Mechanisms of MDSC Activation

Activated T cells

Tumor and Stromal Cells

Signal transducer and activator of transcription (STAT)
MDSC Induction

- Tumor (Growth factors/Cytokines)
- Traumatic Stress
- Autoimmunity
- Parasite Infections
- Viral Infections
- Bacterial Infections
MDSC – Mechanisms of Suppression

Tumor Progression

Granulocytic subset
ROS, Arginase
Monocytic subset
NO, Arginase

MDSC – Arginase, iNOS

Induce
Treg

Macrophage M2
Cell ↓ IL-12

IL-10

Th2
Cell

Reduce Homing to lymph tissue and Tumor

CD62L (L-selectin)

Arginine Reduction
Cysteine Reduction

ROS
NO
(H2O2, peroxynitrate)

Nitrate TCR

Apoptosis

CD4
T cell

CD8
T cell

Cell Cycle Arrest

CD3ζ↓ CD4 CD4
T cell

CD3ζ↓ CD8 CD8
T cell

CD3ζ
T cell

MDSC – Mechanisms of Suppression

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Cysteine Reduction

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Reduce Homing to lymph tissue and Tumor

CD62L (L-selectin)
## Markers Expressed by Murine and Human MDSC

<table>
<thead>
<tr>
<th></th>
<th>Murine Monocytic-MDSC</th>
<th>Granulocytic-MDSC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gr1</strong></td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td><strong>CD11b</strong></td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td><strong>F4/80</strong></td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td><strong>Ly6C</strong></td>
<td>(+hi)</td>
<td>(+low)</td>
</tr>
<tr>
<td><strong>Ly6G</strong></td>
<td>(-)</td>
<td>(+)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Human Monocytic-MDSC</th>
<th>Granulocytic-MDSC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD33</strong></td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td><strong>CD11b</strong></td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td><strong>CD66b</strong></td>
<td>(+/-)</td>
<td>(+)</td>
</tr>
<tr>
<td><strong>CD14</strong></td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td><strong>CD15</strong></td>
<td>(+/-)</td>
<td>(+)</td>
</tr>
<tr>
<td><strong>CD124</strong></td>
<td>(+)</td>
<td>(+)</td>
</tr>
</tbody>
</table>
Ly6G$^{hi}$ Neutrophilic MDSC are Gr1$^{hi}$ and Ly6G$^{lo}$ Monocytic MDSC are Gr1$^{lo}$
MDSC Isolated from RCC Patient’s Tumor

A.

B.
### Targeting MDSC To Improve Immunotherapy

<table>
<thead>
<tr>
<th>Agents</th>
<th>Mode of Action</th>
<th>Tumor Type</th>
<th>MDSC Reduction (numbers/function)</th>
<th>T cell Response Improved</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VEGF Trap</strong> (Fusion Protein)</td>
<td>Binds VEGF</td>
<td>Multiple Types</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><strong>Anti-VEGF</strong> (bevacizumab)</td>
<td>Binds VEGF</td>
<td>RCC</td>
<td>Mixed (1 yes, 1 NO)</td>
<td>Not Clear</td>
</tr>
<tr>
<td><strong>TKI</strong> (AZD2171)</td>
<td>Blocks VEGFR Signaling</td>
<td>Multiple Types</td>
<td>Slight Reduction in Numbers</td>
<td>Not Tested</td>
</tr>
<tr>
<td><strong>Triterpenoids</strong> (CDD0-Me)</td>
<td>Antioxidant Reduced ROS</td>
<td>RCC</td>
<td>No reduction #</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Phosphodiesterase-5</strong> (Sildanefil)</td>
<td>Reduces Arginase 1 &amp; NOS-2 expression</td>
<td>Head/Neck Myeloma</td>
<td>Reduced Function</td>
<td>Yes</td>
</tr>
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Targeting MDSC To Improve Immunotherapy

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</tr>
</thead>
<tbody>
<tr>
<td>All-Trans retinoic Acid</td>
<td>MDSC Differentiation</td>
<td>mRCC</td>
<td>Reduced Numbers</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>(Increased glutathione synth and reduced ROS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin D3</td>
<td>Promote Differentiation</td>
<td>Head &amp; Neck</td>
<td>Reduced numbers</td>
<td>Yes</td>
</tr>
<tr>
<td>Bioactive Metabolite</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gencitabine + Cyclophosphomide</td>
<td>Chemotherapeutic Drug</td>
<td>Breast Cancer</td>
<td>Reduced numbers</td>
<td>?</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>TKI</td>
<td>mRCC</td>
<td>Reduced Numbers</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>(blocks Proliferation of mMDSC and causes Apoptosis of nMDSC)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Multitargeted Approaches in mRCC: Sunitinib (SU11248)

- Small-molecule receptor tyrosine kinase inhibitor\(^1\)
- Inhibits all VEGFRs, PDGFR-A, PDGFR-B, c-KIT, and FLT-3\(^1\)
- Oral administration\(^1\)
- Both antitumor and antiangiogenic activity\(^1\)
- FDA approved January 26, 2006 for treatment of advanced RCC\(^2\)
- 50% response rate in mRCC

Sunitinib reverses MDSC accumulation in mRCC patients

Sunitinib-mediated improvements in T cell function are reproduced by *in vitro* MDSC depletion.

- **mRCC Pre-MDSC Immunodepletion**
  - CD15 PE vs. CD14 APC
  - CD3 APC vs. IFNγ FITC
  - 6.59%

- **mRCC Post-MDSC Immunodepletion**
  - CD15 PE vs. CD14 APC
  - CD3 APC vs. IFNγ FITC
  - 14.97%

- **Comparison Bar Graph**
  - AMN n = 4
  - mRCC n = 8
  - mRCC -MDSC n = 8
  - IFNγ and IL-4 cytokines

Tumor Associated Macrophages

**M1**
- **Stimuli**
  - LPS
  - IFNγ/TNF
- **Functions**
  - MHCII
  - IL-1
  - TNF
  - IL-6
  - IL-23
  - IL-12
  - IL-10
  - iNOS
- **Responses**
  - DTH
  - Type 1 Inflammation
  - Th1 Responses
  - Tumor Resistance

**M2**
- **Stimuli**
  - Immune Complexes + IL-1/LPS
  - CSF-1
  - IL-4, IL-10
  - IL-13
- **Functions**
  - MHCII
  - Arg
  - TGFβ
  - IL-10
  - IL-1ra
  - Th2 Responses
  - Tumor Promotion
  - Type II Inflammation
  - Allergy
Obstacle To Overcome To Promote Immunotherapy in Cancer
Future Directions

• Identify new targets for reducing Treg numbers and/or their suppressive function.

• Better understand the role of other immune suppressive T cell populations (Tr1/Tr3, CD8) in tumor-induced immune suppression. Identify targets for blocking/deleting them.

• Identify which of the various strategies shown to reduce MDSC in the peripheral blood of patients are also effective within the tumor microenvironment and define which ones promote strong anti-tumor immunity.

• In clinical studies test whether effective blocking of Tregs and MDSC will provide greater efficacy for different forms of immunotherapy (vaccines and adoptive therapy).