Defining The Role of TKI in Reducing Immune Suppression While Improving T cell Responsiveness and Efficacy of Immunotherapy For the Treatment of Tumors

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Immune Dysregulation in Cancer

- Increased suppression of a Th1 immune response and increased Th2 cytokine bias
- Increased apoptosis of T lymphocytes
- Impaired DC maturation and function
- Observed in many tumor types including: RCC, Gliomas (GBM), Squamous cell carcinoma of head and neck, Ovarian and Melanoma.
Reduced T cell IFNγ response in RCC patients

- Normal POMC: n=18
- mRCC POMC: n=43
- TILS: n=13

p<0.0001

p<0.0001

p=0.187
Sunitinib-mediated modulation of tumor-induced immune suppression

- Sunitinib is a small molecule receptor tyrosine kinase inhibitor which was designed to limit angiogenesis.

- It promiscuously targets VEGFR, ckit (SCF receptor), flt3, PDGFR, and M-CSFR receptor tyrosine kinases (rTKs).

- Sunitinib has major therapeutic impact against renal cell carcinoma and ckit\textsuperscript{pos} GIST tumors.
Sunitinib Modulation of Immune Cells

• Sunitinib reverses immune suppression and decreases T-regulatory cells in RCC patients. Finke J et al Clinical Cancer Research 2008

• Sunitinib-induced myeloid lineage redistribution in RCC patients: CD1c dendritic cell frequency predicts progression-free survival. Van Cruijsen H. Clinical Cancer Research 2008

• The novel role of tyrosine kinase inhibitor in the reversal of immune suppression and Modulation of tumor microenvironment for immune-based cancer therapies. Ozao-Choy J et al Cancer Research 2009


• Sunitinib mediates reversal of myeloid-derived suppressor cell accumulation in renal cell patients carcinoma Ko J et al Clinical Cancer Research 2009
Sunitinib reverses MDSC accumulation in mRCC patients

IFN-γ Levels in Stimulated PBMCs from sunitinib treated mRCC Patients

Patient groups:
- Normals (n=21)
- Pre-Treatment (n=45)
- C1D28 (n=44)
- C2D28 (n=25)
- C4D28 (n=15)

Comparisons:
- Pre-Treatment vs. Normals: p<.0005/.0005
- C1D28 vs. Normals: p=.0018/.0054
- C2D28 vs. Normals: p=.0013/.17
- C4D28 vs. Normals: p=.010/.026

MDSC Levels in PBMCs

Patient groups:
- Normals (n=10/10)
- Pre-Treatment (n=28/27)
- C1D28 (n=25/23)
- C2D28 (n=17/16)
- C4D28 (n=18/18)

Comparisons:
- Pre-Treatment vs. Normals: p=.0012/.0084
- C1D28 vs. Normals: p=.0018/.0054
- C2D28 vs. Normals: p=.0013/.17
- C4D28 vs. Normals: p=.010/.026
MDSC decline is associated with T effector IFNγ recovery with Sunitinib Treatment
Sunitinib-mediated improvements in T cell function are reproduced by \textit{in vitro} MDSC depletion

\textbf{mRCC Pre-MDSC Immunodepletion} \hspace{1cm} \textbf{mRCC Post-MDSC Immunodepletion}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image.png}
\caption{Comparison of CD3, CD14, IFN$\gamma$, and IL-4 expression in T cells before and after MDSC depletion.}
\end{figure}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|}
\hline
\textbf{Condition} & \textbf{% Cytokine+ T Cells} \\
\hline
AMN \hspace{0.5cm} n = 4 & \hspace{1cm} \textbf{p < .05} \\
\hline
mRCC \hspace{0.5cm} n = 8 & \hspace{1cm} \textbf{p < .05} \\
\hline
mRCC -MDSC \hspace{0.5cm} n = 8 & \\
\hline
\end{tabular}
\caption{Cytokine expression in T cells post-MDSC depletion.}
\end{table}
Sunitinib modulates MDSC suppressive effect in vitro

- *p < 0.12 versus T+MDSC
- † p < 0.007 versus T+MDSC

**Diagram**

- T cells alone
- T+MDSC
- T+MDSC sunitinib 0.1ug/mL
- T+MDSC sunitinib 1.0ug/mL
- T+MDSC L-arginine 2mM
- T+MDSC catalase 200U/mL

**Bar Graph**

- %IFN+ in CD3+

**Statistical Notes**

- p = 0.002
- * p < 0.12 versus T+MDSC
- † p < 0.007 versus T+MDSC
Sunitinib induces myelo-specific apoptosis and patient MDSC-specific apoptosis at 1ug/mL
Conclusions:

• Sunitinib mediates reversal of MDSC accumulation in RCC patients and thereby restores patient T cell function.

• Sunitinib has a toxic, rather than DC-differentiating effect on RCC patient MDSC in vitro, which may account for its partial inhibition of MDSC suppressive effect in vitro.

• Sunitinib-mediated MDSC declines in RCC patients were not correlated with changes in tumor volume.
Sunitinib significantly reverses MDSC-mediated immune suppression in mice bearing RCC and non-RCC tumors.
Sunitinib significantly reverses MDSC-mediated immune suppression in mice bearing RCC
Sunitinib-mediated MDSC decline may not be attributed to single target
Method to evaluate sunitinib’s impact on MDSC expansion in vivo

**In vivo BrdU assay**

- **9 days sunitinib treatment**
- **s.c. tumor cell injection**
- **4h i.p. BrdU pulse**
- **Sac mice- spleen, BM, tumor harvest and BrdU staining for FACS**
Ly6G$^{hi}$ Neutrophilic MDSC are Gr1$^{hi}$ and Ly6G$^{lo}$ Monocytic MDSC are Gr1$^{lo}$

Gr1$^{hi}$ N-MDSC = green, Gr1$^{lo}$ M-MDSC = blue
Sunitinib inhibits pathological proliferation of M-MDSC in the spleen but not in the bone marrow.

In vivo BrdU uptake of MDSC subsets in spleen

- Naïve Gr1lo
- Naïve Gr1hi
- 4T1 Gr1lo
- 4T1 Gr1hi
- 4T1+Sut Gr1lo
- 4T1+Sut Gr1hi

In vivo BrdU uptake of MDSC subsets in bone marrow

- Naïve Gr1lo
- Naïve Gr1hi
- 4T1 Gr1lo
- 4T1 Gr1hi
- 4T1+Sut Gr1lo
- 4T1+Sut Gr1hi

Day 3 Day 6 Day 9

Days following sunitinib initiation

Day 3 Day 6 Day 9

Days following sunitinib initiation

11% 38% 13% 52%

Gr1 hi Neutrophilic MDSC
Gr1 dim Monocytic MDSC
Sunitinib impairs N-MDSC viability in vivo and in vitro

AnnexinV binding of splenic N- MDSC in vivo

Days following sunitinib initiation

Viability of MDSC in vitro
Conclusions:

• Similar to the human studies, sunitinib treatment reduces MDSC levels and restored T cell response in several mouse tumor models.

• Sunitinib inhibits the pathological expansion in the spleen of proliferative Gr1lo M-MDSC.

• Sunitinib has an apoptotic, rather than DC-differentiating effect on N-MDSC.
Sunitinib’s impact on splenic MDSC is independent of sunitinib’s demonstrable anti-tumor effect.

**Renca tumors are extremely sensitive in vivo**

**4T1 tumors are relatively insensitive in vivo**
Tumor-associated MDSC in resistant 4T1 tumor model are relatively resistant to sunitinib.

Mild decrease in tumor bed MDSC in treated 4T1 mice.

Type 1 function of Tumor Infiltrating T cells remains suppressed.

Graph showing changes in myeloid cells and IFN-γ+ T cells in naive, 4T1, and 4T1+Sut conditions.
GM-CSF uniquely protects MDSC in the presence of sunitinib
GM-CSF is selectively expressed in tumor microenvironment in vivo

Proteome Profile Array

Luminex Array

4T1 Plasma

4T1 + Sunitinib Plasma

4T1 Tumor

4T1 + Sunitinib Tumor

G-CSF | GM-CSF

Luminex Array

4T1 Plasma

4T1 Spleen

4T1 Tumor

No Treatment | Sunitinib Treatment

p<.00003
Conclusions:

• Sunitinib-mediated MDSC declines in RCC patients and TB-mice are direct and independent of anti-tumor effects or consequent changes in cytokines.

• GM-CSF may mediate intratumoral resistance to sunitinib in RCC patients and TB-mice.
Combining Sunitinib with Immunotherapy
CT26 injected s.c.

CpG(ODN1826, 50µg)/pIC(50µg) (d10-27)
Twice weekly
Intra-tumor

Sunitinib i.p.
40 mg/kg

Monitor Tumor Growth
Significantly Improved Survival is Associated With Combined Sunitinib and Immunotherapy (CpG and pIC)

Peter Cohen MD, Mayo Clinic Arizona
Collaborators at University of Pittsburgh Medical Center

Anamika Bose, Ph.D.  Walt Storkus, Ph.D.
**Combination Therapy Design**

**Vaccine**
- BM-DC (5d)
- Infect Ad.IL12 (100 MOI), 48h
- OVA CTL + Th Peptide Pulse 10 µM, 37°C, 4h

**Timepoints**
- 0
- 7
- 14
- 21

**MO5 (B16.OVA)**
- Tumor s.c. left flank

**Key Cohorts**
- Sunitinib (oral, 1 mg/d x 7d)
- Vaccine (10⁶ DC/peptide, s.c. flank opposite tumor)

**Monitor growth, IHC, Phenotype (MDSC/Treg) T cell Assays**
Combination vs. Single Modality Therapy of Day 10 Established M05 (B16.OVA) Melanomas with Sutent and Specific Vaccination

MO5 Tumor Volume (mm³)

Days Post Tumor Inoculation

(number tumor-free mice day 24)
SUTENT +/- Vaccine Immunomonitoring: TIL (d24)

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Sutent</th>
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<tbody>
<tr>
<td>No</td>
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<tr>
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<td>d7-13</td>
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<tr>
<td>d7/14</td>
<td>d14-20</td>
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**IFN-γ (ng/ml)**

- **No**: 3
- **d7**: 2
- **d7/14**: 1.3
- **d14**: 15.2
- **d7-13**: 1.5
- **d14-20**: 1.8

**H-2Kb/OVA**

- **T Only**: 1.3
- **T/DC**: 2.2
- **T/DC/OVA**: 1.9
- **CD8**: 2.5
- **CD11b**: 4.4
- **Gr1**: 9.4
- **CD4**: 8.3
- **Foxp3**: 6.7
- **Sutent**: 15.2

**Tetramer CD8**

- **CD8**: 1.3
- **CD11b**: 2.2
- **Gr1**: 1.9
- **CD4**: 2.5
- **Foxp3**: 4.4
- **Sutent**: 9.4
- **T/DC**: 8.3
- **T/DC/OVA**: 6.7
- **Gr1**: 15.2

**Gr1**

- **CD8**: 8.9
- **CD11b**: 2.3
- **CD4**: 6.3
- **Foxp3**: 3.7
- **Sutent**: 2.3
- **T/DC**: 2.7
- **T/DC/OVA**: 5.9
- **Gr1**: 6.0
- **Sutent**: 1.5

**CD4**

- **CD8**: 4.6
- **CD11b**: 0.8
- **CD4**: 3.9
- **Foxp3**: 3.3
- **Sutent**: 3.1
- **T/DC**: 1.4
- **T/DC/OVA**: 1.7
- **Gr1**: 2.4
- **Sutent**: 1.8
Conclusions

• SUTENT/sunitinib improves anti-tumor efficacy when combined with specific immunization as a combinational therapy.

• Combinational therapy associated with reductions in MDSC and Treg frequencies in the TME

• Therapeutic benefits correlated with vaccine-induced CD8+ TIL frequencies (tetramer)
Combinational Therapy

- Sutent, 50mg/daily

- Vaccinate with Type-1-polarized dendritic cell s(aDC-1) loaded with a mixture of 3 RCC-associated T-helper peptide epitopes in HLA-DR4+ patients with mRCC. 
  EphA2\textsubscript{53-75}, G250\textsubscript{249-268}, MAGE-6\textsubscript{140-160}

- RCC expression in situ: EphA2 (97%), G25 (85%+) and MAGE-3/6 (>80%)

- Evaluate changes in the magnitude and function polarization of RCC antigen-specific CD4+ (and CD8+) T cells in the peripheral blood.
Collaborators

- Dr. Peter Cohen MD
- Dr. Walter Storkus PhD
- Dr. Rini MD
- Dr. Bukowski MD
- Dr. Charles Tannenbaum PhD

Dr. Jennifer Ko (MD/PhD), Joanna Ireland, Patricia Rayman, Dr. Kausik Biswas (PhD), Soumika Biswas, Leticia Varella (MD), Cynthia Hilston, Yuntao Li.

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