Unraveling the Basic Components of Cancer Immunotherapy

Alan L. Epstein MD, PhD
Department of Pathology
USC Keck School of Medicine
Working Hypothesis

• Targeting missing immunostimulatory molecules to tumor can generate complete immune response with memory

• Deletion of natural immunosuppression can enable immunotherapy to be effective
Targeting Tumor Necrosis with TNT Antibodies

USC
Major Characteristics of TNT Antibodies

- Recognize abundant intranuclear antigens present in all cancers, all species
- Have long retention times in tumor
- Have enhanced uptake after cytoreductive therapies
- Localize to necrosis, a site rich in tumor antigens
Tissue Biodistribution of I-125-chTNT-3/B in ME-180 Carcinoma-bearing Nude Mice

% dose/gram

1 day
3 days
5 days
7 days
10 days

organ

blood muscle heart lung liver stomach kidney tumor
TNT Antibody Uptake in Tumor

OLD NECROSIS
NEW NECROSIS
VIABLE CORDS

Colon 26 subcutaneous tumor

Bronchioalveolar Lung Carcinoma

LUNG TUMOR
INFLAM 9 x 10cm
MET
Macroautoradiography of $^{125}$I-TNT-1 in ME-180 Human Cervical CA

6 hr

2 days

10 days

kidney

liver
Macro and Microautoradiography of $^{125}\text{I}$-TNT-3

Necrotic zone

Viable zone
Enhanced Uptake of TNT in Taxol Treated Colon 26 Tumors

% dose/gram

- I-125 TNT
- I-125 TNT after 1 day pretx with Taxol; (20mg/Kg)
- I-125 TNT after 2 days pretx with Taxol; (20mg/Kg)
- I-125 TNT after 3 days pretx with Taxol; (20mg/Kg)

Organ:
- Blood
- Lung
- Liver
- Kidney
- Tumor
Methods of Immunotherapy

- Vaccines
- Cytokine Therapy
- Adoptive Transfer of Immunity
- Fusion Proteins
  - Targeted (MAb)
  - Untargeted (Fc)
- Genetic alteration of T-cells
- Immunomodulatory drugs
Targeted Fusion Proteins

C-Terminal Fusion

N-Terminal Fusion

Chemokines, B7

Cytokines, Type II costimulatory molecules

Extracellular domains

Fc portion of human IgG1

Untargeted Fc
Cytokine Fusion Proteins

IL-2, IL-4, IL-12, TNFα, GM-CSF, IFNγ

Chemokine Fusion Protein

LEC
Immunotherapy of MAD 109 Lung CA Using Immunocytokine Fusion Proteins

- no treatment
- IL-2 (20ug)
- IL-2 (20ug) + muINFg (20ug)
- IL-2 (20ug) + TNFa (20ug)
- IL-2 (20ug) + muGM-CSF (5ug)
- IL-2 (20ug) + muINFg (20ug) + TNFa (20ug)
- IL-2 (20ug) + muINFg (20ug) + GM-CSF (5ug)
LEC Chemokine

- Liver Expression Chemokine (LEC)
- A CC family ($\beta$ family) chemokine (CCL16)
- Located on chromosome 17q in CC cluster
- Chemoattracts PMNS, monocytes, dendritic cells, and lymphocytes
- Interacts with CCR1, CCR5, and CCR8 receptors
LEC/chTNT-3 Immunotherapy in 3 Tumor Models of the BALB/c Mouse

Colon 26 Colon Carcinoma

- PBS
- LEC/chTNT-3 (20ug)

RENCA Renal Carcinoma

- PBS
- LEC/chTNT-3 (20ug)

MAD 109 Lung Carcinoma

- PBS
- LEC/chTNT-3 (20ug)
Histologic and IHC Analysis of Tumor Sections

H&E

Dendritic Cells

PMNS

Control Treated  LEC/chTNT-3 Treated
Lymphocyte Depletion Studies

- **CD4⁺ T cell depletion**: GK1.5 (0.5mg ip q5 days)
- **CD8⁺ T cell depletion**: 2.43 (0.5 mg ip q5 days)
- **NK depletion**: anti-asilao GM1 (0.35mg ip q5 days)
- **CD4⁺CD25⁺ depletion**: PC61 (0.5 mg ip Day 0)

After Depletion

<table>
<thead>
<tr>
<th>CD4</th>
<th>CD8⁺</th>
<th>CD25 (7D4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After Depletion</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Control

After Depletion
T-cell Subset Depletion Studies in Colon 26

The graph shows the volume (cm³) over time (Days) for different conditions:

- chTNT-3
- CD4 depleted +LEC/chTNT-3
- CD8 depleted +LEC/chTNT-3
- NK depleted +LEC/chTNT-3
- CD4 depleted
- CD8 depleted
- NK depleted
- LEC/chTNT-3

The conditions are compared over a range of days from 5 to 17 for the left graph and from 7 to 19 for the right graph.
Cell Proliferation Assay of TDLN after Incubation with Tumor Lysates

![Graph showing cell proliferation assay results for different conditions.](image-url)
Tumor Re-challenge Studies (3 months)

A: Colon 26 Naïve Mice

B: Colon 26 Regressed Mice

MAD109
Combination Cytokine or Chemokine Fusion Protein Immunotherapy and T-cell Subset Depletion in Colon 26

<table>
<thead>
<tr>
<th>Immunotherapy</th>
<th>T-cell Subset Depletion</th>
<th>% Tumor Reduction (Day 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>chTNT -3 (control)</td>
<td>-</td>
<td>0%</td>
</tr>
<tr>
<td>chTNT -3 (control)</td>
<td>CD4⁺ depletion</td>
<td>33%</td>
</tr>
<tr>
<td>LEC/chTNT -3</td>
<td>-</td>
<td>60%</td>
</tr>
<tr>
<td>LEC/chTNT -3</td>
<td>CD4⁺ depletion</td>
<td>100%</td>
</tr>
<tr>
<td>chTNT -3/IL -2</td>
<td>-</td>
<td>38%</td>
</tr>
<tr>
<td>chTNT -3/IL -2</td>
<td>CD4⁺ depletion</td>
<td>64%</td>
</tr>
<tr>
<td>chTNT -3/IFN -γ</td>
<td>-</td>
<td>32%</td>
</tr>
<tr>
<td>chTNT -3/IFN -γ</td>
<td>CD4⁺ depletion</td>
<td>33%</td>
</tr>
<tr>
<td>chTNT -3/TNF -α</td>
<td>-</td>
<td>10%</td>
</tr>
<tr>
<td>chTNT -3/TNF -α</td>
<td>CD4⁺ depletion</td>
<td>33%</td>
</tr>
</tbody>
</table>

1 Antibodies and fusion proteins (20 ug/dose) were injected iv for 5 consecutive days after tumors reached 0.5cm in diameter.

2 CD4⁺ depletion (0.5 mg/dose of GK1.5) was performed ip 1 day after tumor implantation and repeated every 5 days.
* The concept of suppressor T cells was elusive until: Sakaguchi et al identified a subpopulation (about 10%) of CD4+ cells that express CD25.

* Most cell markers for Treg cells are also expressed on CD4+CD25− cells upon activation.

* None of the known cell surface markers appear to be responsible for CD4+CD25+ mediated suppression.
Real-Time PCR Analysis of Foxp3 in 4 Treated and Untreated Murine Tumor Models

**Colon 26**

- Tumor
- Treated tumor
- LN
- Treated LN

**RENCA**

- Tumor
- Treated Tumor
- LN
- Treated LN

**MAD109**

- Tumor
- Treated Tumor
- LN
- Treated LN

**4T1**

- Tumor
- Treated Tumor
- LN
- Treated LN

**Y Axis:** Fold Increase over control
Untargeted and Targeted Co-stimulation

B7

GITRL
Co-stimulatory Molecules

T-cell

- Co-stimulatory Molecules
- Inhibitory signal
- T-cell
- Dendritic cell

- CD8
- CD4
- CD80 (B7.1)
- CD86 (B7.2)
- CD154
- CD40
- GITR
- TCR
- MHC class I
- MHC class II
- Peptide
- CD28
- CTLA4
- CD137
- OX40
- GITRL
- CD137L
- OX40L
- CD40
B7.1-Fc

Non-reducing B7.1-Fc

Reducing B7.1-Fc

202 KD
133 KD
71 KD
41.8 KD
30.6 KD

B7.1/NHS76

Reducing NHS76  Reducing B7.1/NHS76

H

L

103 KD
77 KD
50 KD
34.3 KD
28.8 KD
20.7 KD

SDS PAGE
CFSE Proliferation Assay

NHS76  B7.1/NHS76  B7.1/Fc

anti-CD3 alone  anti-CD3 + B7.1/NHS76  anti-CD3 + B7.1/Fc
B7.1-Fc Dosing Study in Colon 26 Tumor Model

Tumor volume (cm^3)

Days after tumor implantation

- Isotype control
  - Ab 40µg
- B7.1-Fc 40µg
- B7.1-Fc 20µg
- B7.1-Fc 10µg
- B7.1-Fc 5µg
- B7.1-Fc 1µg
- B7.1-Fc 0.5µg

treatment
IHC of Control and Treated Colon 26

<table>
<thead>
<tr>
<th>H &amp; E</th>
<th>Control</th>
<th>B7.1-Fc</th>
<th>B7.1-Fc + CD25 depletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Tumor Infiltrating Lymphocytes (TIL)

CD11b+

Control

B7.1-Fc

4.59%

12.73%

CD11c+

CD25+ Depletion

B7.1-Fc + CD25+ Depletion

6.06%

11.23%
Activation of TIL With Tumor Lysate In Vitro

Control

CD25+ Depletion

B7.1-Fc

B7.1-Fc + CD25+ Depletion
T-Cell Depletion Studies in B7.1-Fc Treated Colon 26-Bearing Mice

Graph showing tumor volume (cm³) over days after tumor implantation, with treatments including:
- Isotype control Ab NHS76 30ug
- B7.1-lg 10ug
- B7.1-lg 10ug + CD4 depletion
- B7.1-lg 10ug + CD8 depletion
- B7.1-lg 10ug + CD25 depletion

Arrows indicating treatment times.
IFN-gamma Vital for B7.1 Therapy as Demonstrated in KO mice

Mechanism of Action Studies

Anti IL-4 Therapy Does Not Reverse B7.1-Fc Therapy
Dual Function of GITR

Responder T Cell

TCR

GITR

MHC

Ag

Costimulation

Abrogation of Suppressive Function

Treg Cell

APC

TCR

GITR

MHC

Ag

APC
Activity Assay of GITRL Fusion Proteins at 48 Hours

- Performed on naïve splenocytes.
- 2ug of protein was used for each sample.
- CFSE stained CD4⁺ T cells

**CD3 alone**
- M2 = 11%

**DTA-1**
- M2 = 45%

**Fc-GITRL**
- M2 = 50%

**TNT3/ GITRL**
- M2 = 55%
Targeted and Non-targeted GITRL Dosing Studies in Colon 26 Tumor Model

**Fc-GITRL**
- 130 nM mTNT-3
- 130 nM Fc-mGITRL
- 275 nM Fc-mGITRL
- 500 nM Fc-mGITRL
- 1 uM Fc-GITRL
- 3 uM DTA-1 Antibody

**TNT-3/GITRL**
- 130 nM mTNT-3
- 130 nM mTNT3/mGITRL
- 275 nM mTNT3/mGITRL
- 550 nM mTNT3/mGITRL
- 1 uM mTNT3/mGITRL

T-cell Subset Deletion Studies
- 90ug LFA/Fc
- 90ug Fc/GITRL
- 90ug DTA-1
- 0.5mg anti-CD4
- 0.5mg anti-CD4+90ug Fc/GITRL
- 0.5mg anti-CD8
- 0.5mg anti-CD8+90ug Fc/GITRL

Days Post Tumor Implantation
H & E of GITRL Treated COLON-26 Bearing Mice

Control

1uM TNT-3/GITRL

Vessel thrombosis

Cell necrosis

Necrosis

1uM TNT-3/GITRL
Targeting Innate Immunity

TNT-3/CpG
Multiple Functions of CpG

- Potential for CpG ODNs
  - Protective Immunity
    - TLR9 detects CpG → triggers ↑ response
  - Allergies
    - TH1 response
  - Vaccine Response
    - Th1 and pro-inflammatory cytokines → Improves APC function
    - Promotes induction of Ag-specific response
  - Cancer Therapy
    - ↑ CTLs and NK cells
Heterobifunctional Linkage of CpG to Antibody

\[
\text{Sulfo-EMCS} + \text{MAb - NH}_2 \rightarrow \text{MAb - NH}_2
\]

\[
\text{DMTrO-(CH}_2)_3-S-S-(CH}_2)_3-O-Succinyl \quad \text{CpG}
\]

\[
\text{3'-Thiol Modifier C3}
\]

\[
\text{CpG - succinyl - O-(CH}_2)_3-S \quad + \quad \text{MAb - NH}_2 \rightarrow \text{MAb}
\]

\[
\text{CpG - succinyl - O-(CH}_2)_3-S \quad \text{CpG/MAb}
\]
In Vitro Assay Demonstrating CpG Activity of Immunoconjugate

IL-6 J77743A Cells

Concentration (pg/mL)
chTNT-3/CpG Immunotherapy

- chTNT-3 (30ug/dose) iv
- chTNT-3/CpG1745 (30ug/dose) iv
- chTNT-3/CpG1826 (5ug/dose) iv
- chTNT-3/CpG1826 (30ug/dose) iv
- CpG1826 (5ug/dose) intratumoral

Days: 5 7 9 11 13 15 17 19 21 23 25

Tumor Size (cm³)
SUMMARY: Major Pathways of Immune Activation for Cancer Immunotherapy

- Chemotaxis (chemokines)
- Co-Stimulation (second signal)
- Combination T-cell activation and inhibition of Treg (GITRL)
- Activators of innate immunity (CpG)
**SUMMARY:** Major Inhibitory Mechanisms That Generate Tolerance to Tumors

- Treg cells
- T-cell death receptors (PD-1, 2)
- Soluble cytokines (IL-10, TGFβ)
- Inhibition of CD28 Co-stim (CTLA-4, B7.1-Fc?)
- IDO (Indoeamine 2,3-dioxygenase)
  - degrades tryptophan
- Loss or release of MHC class I molecules