T Cell Immunotherapy - Optimizing Trial Design

Office of Biotechnology Activities

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Target selection

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A critical challenge confronting the development of human cancer immunotherapy is the identification of antigens to target

1. Differentiation antigens overexpressed on cancers compared to normal tissue (MART-1, gp100, CEA, Her-2)

2. Antigens expressed on cancers and on non-essential normal tissues (CD19, thyroglobulin)

3. Shared antigens unique to cancer (cancer-testes antigens)

4. Mutations unique to each cancer (EGFRvIII)

5. Critical components of the tumor stroma (VEGFR2, FAP)
# TCR Gene Therapy in Patients with Metastatic Melanoma

<table>
<thead>
<tr>
<th>TCR</th>
<th>Response Total</th>
<th>OR (%)</th>
<th>Skin (Grade 1/2/3)</th>
<th>Uveitis (Grade 1/2/3)</th>
<th>Auditory (Grade 1/2/3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MART-1TCR (DMF5)</td>
<td>20</td>
<td>6(30%)</td>
<td>11/3/0</td>
<td>2/9/0</td>
<td>2/0/7</td>
</tr>
<tr>
<td>gp100TCR (gp154)</td>
<td>16</td>
<td>3(19%)</td>
<td>11/4/0</td>
<td>0/4/0</td>
<td>2/2/3</td>
</tr>
<tr>
<td>(Total)</td>
<td>36</td>
<td>9(25%)</td>
<td>22/7/0</td>
<td>2/13/0</td>
<td>4/2/3</td>
</tr>
</tbody>
</table>

Grade 1
- Skin: erythema
- Eye: no symptoms
- Ear: 15-25dB, 2 freq.

Grade 2
- Skin: desquamation <50%
- Eye: anterior, steroid drops
- Ear: >25dB, 2 freq.

Grade 3
- Skin: desquamation >50%
- Eye: pan uveitis
- Ear: >25dB, 3 freq.

(Science 314:126, 2006; Blood 114:535, 2009)
Carcinoembryonic Antigen (CEA)

CEA is a highly glycosylated protein:

- 50% carbohydrate
- 180 kDa

Highly expressed in fetal development with very low expression in colonic epithelial cells, squamous epithelium in the esophagus and cervix, epithelial cells of cervix

High expression in some cancers:
- colorectal
- breast
- lung
- cervix
- stomach
- pancreas
- and others
HLA-A2 transgenic mouse T cell clone specifically reactive with human CEA:691-699

IFN$\gamma$ (pg/ml)

Peptide reactivity

- T2 + control peptide
- T2 + CEA:691-699
- media
- SW620
- SW480
- SW403
- COS-A2-ESO
- 293-A2-p53
- H508
- SW1463
- COS-A2-CEA
- 293-A2-CEA

HLA-A2$^+$/CEA$^-$/weak

HLA-A2$^+$/CEA$^+$

Peptide reactivity HLA-A2$^+$/CEA$^-$/weak HLA-A2$^+$/CEA$^+$

>140,000 >140,000 28,239 >140,000
Serum CEA (% pre-Rx level)

Days (relative to cell infusion)

Patient 1

Patient 2

Patient 3

(223)

(48)

(3.8)
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5. Critical components of the tumor stroma (VEGFR2, FAP)
Cancer/Testis Antigens Expressed in Multiple Tumor Types

% of positive tumor by RT-PCR

Tumor Type

Bladder  NSCLC  Melanoma  Ovarian  Hepatocellular  Myeloma  Squamous cell carcinoma

MAGE-A3  MAGE-A1  NY-ESO-1
MAGE-TCR patient Bx-IHC staining for MAGE-A

Patient: MS

11-5784; neurons negative for MAGE

Patient: GT

11-5700; few neurons and axons positive for MAGE
A critical challenge confronting the development of human cancer immunotherapy is the identification of antigens to target.

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EGFRvIII Activating Mutation is an Excellent Target for the Treatment of Glioblastoma

Expressed in 30-50% of glioblastomas

Not expressed in normal tissues

Likely essential for the malignant phenotype so loss variants are unlikely

Highly specific antibodies that recognize EGFRvIII are available to produce CAR for use in cell transfer therapy
# Recognition of Glioblastoma by T-cells Expressing an anti-EGFRvIII Chimeric Antigen Receptor

<table>
<thead>
<tr>
<th>Transduction</th>
<th>Media</th>
<th>U251</th>
<th>U251</th>
<th>Glioblastoma Stem Cell Lines*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EGFRwt</td>
<td>EGFRvIII</td>
<td>1228</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>308</td>
</tr>
<tr>
<td>GFP</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>882</td>
</tr>
<tr>
<td>EGFRvIII CAR</td>
<td>384</td>
<td>331</td>
<td>4523</td>
<td>3306</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>4406</td>
</tr>
</tbody>
</table>

*All lines express EGFRvIII  
(R. Morgan, H. Fine et al)
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Rationale for the anti-VEGFR2 Gene Therapy Protocol

Vascular endothelial growth factor (VEGF) stimulates tumor angiogenesis by binding to its receptor, VEGFR2, that is overexpressed on tumor vasculature.

Redundancy in angiogenic pathways limits the effectiveness of therapy with anti-VEGF antibodies.

Destruction of cells bearing VEGFR2 may more effectively destroy tumor vasculature and result in effective cancer treatment.
Anti-VEGFR2 CAR and IL-12 cotransduced mouse T cells induced regression of multiple types of vascularized tumors in mice without exogenous IL-2 administration.

P values: DC101 CAR/Flexi-IL12 vs no treatment group; DC101 CAR/Flexi-IL12 vs DC101 CAR alone

Personalized immunotherapy using anti-tumor receptor gene-modified lymphocytes

Target tumor antigen determined from sample:
- MART-1
- NY-ESO-1
- SSX-2
- VEGFR2
- VEGFR2
- MAGE-A3
- CD19

Lymphocytes from patient

Viral vector encoding anti-tumor antigen receptor

Anti-tumor lymphocytes

Cells expanded in culture

Cells + IL-2

Reinfused

Tumor sample from patient
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3. Shared antigens unique to cancer (cancer-testes antigens)

4. Shared mutations unique to each cancer (EGFRvIII)

5. Critical components of the tumor stroma (VEGFR2, FAP)

6. Mutations unique to each individual cancer
Mutation frequency (Mutation / Mb)

- Myelodysplasia
- Testis
- Medulloblastoma
- Pancreatic Neuroendocrine
- Renal Carcinoma
- Oligodendroglioma
- Renal cancer
- Hepatocellular Carcinoma
- Ovarian Serous Carcinoma
- Glioblastoma Multiforme
- Pancreatic Cancer
- Ovarian cancer
- Gastric cancer
- Breast cancer
- Colorectal cancer
- Lung Carcinoma
- Melanoma (exons)
- Melanoma (introns)
- Melanoma (14 whole exomes)
- Melanoma (kinome)
Cells reactive with autologous tumor are highly enriched in subsets expressing PD-1, LAG-3, TIM-3 and 41BB.
Cells reactive with autologous tumor are enriched in subsets expressing PD-1, LAG-3, TIM-3 and 41BB.
PD-1+ derived cells are more oligoclonal than PD-1- derived cells.

PD-1-
2985 TCR clonotypes

PD-1+
805 TCR clonotypes
Clones targeting mutated epitopes were found within the 20 most frequent clones in the PD-1+ derived population.
Exomic Sequencing: Potential Clinical Applications

Sequence tumor and matched normal DNA to identify somatic mutations

Utilize HLA binding algorithms to identify candidate epitopes

Stimulate peripheral T cells with mutated candidate peptides *in vitro*

Enrich tumor reactive T cells from TIL using tetramer library of mutated candidate epitopes

Isolate specific T cell receptors

Transduce autologous T cells

Expand, administer to patients