Chemotherapy-Resistant Cell Therapy

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Introduction

- Temozolomide is the most effective chemotherapy for high-grade brain tumors. However, it also kills essential tumor-fighting elements of the immune system.

- Paradoxically, the brain tumor is most vulnerable to immune attack at the time that it is being stressed by chemotherapy. Even Temozolomide-resistant brain tumors show increased surface expression of immunogenic stress-associated molecules until they can repair damage from the chemotherapy. (*PLoS One*, 11Jan 2013)

- Essential components of the immune system can be protected from chemotherapy by drug-resistance immunotherapy (DRI). If drug-resistant cells can be manufactured in large numbers, DRI could be used to treat the patient at the time that the chemotherapy is concentrated in the circulation.

- T-cell receptor (TCR)-γδ T cells are lymphocytes that recognize stress-associated antigens naturally expressed by brain cancer. Following recognition of the tumor, the γδ T cells subsequently and immediately bind and kill the tumor. (*J. Neuro-Oncology*; 101:178 2010)

  - We have developed a cGMP FDA-compliant method to genetically engineer Temozolomide-resistant γδ T cells using an MGMT transgene that protects the cell from the effects of chemotherapy and allows it to remain fully functional in the presence of drug.
TCR-γδ T cells recognize stress-associated antigens naturally expressed by the tumor.

Stress-associated antigens are not expressed on normal astrocytes, therefore normal brain cells are not harmed through this mechanism (J. Neuro-Oncology; 101:178 2010).

Temozolomide chemotherapy increases tumor stress and subsequently a higher density of antigen expression rendering the tumor even more vulnerable to killing by γδ T cells (PLoS One, 11Jan 2013).
In Vivo Testing of $\gamma\delta$ T Cell and Drug Resistant Immunotherapy

Saline-injected control mice

$\gamma\delta$-injected mice (5:1)

Week 2 post-injection

**Effect of $\gamma\delta$ T cells on Induction and Growth of U251flLuc Intracranial Gliomas**

- U251flLuc cells injected alone or with $\gamma\delta$ T cells
- U251flLuc Cells (2.4x10$^6$)
- MST = 21 days
- U251 Cells + $\gamma\delta$ T cells (1.2x10$^6$)
- MST = 48 days

$p < 0.001$
In Vivo Testing of $\gamma\delta$ T Cell and Drug Resistant Immunotherapy

Model:
NSG mice
Inoculated with human neuroblastoma cell line
Treated with TMZ and engineered NK-92 cells

Tumor Inoculation → Treatments → Monitor tumor growth and survival

~ 12 days week 1 week 2 week 3

Percent of Survival

Days after challenge

Drug resistant NK-92 + Chemotherpay
Non-modified NK-92 (chemo+immunotherapy)
untreated
Starting material is peripheral blood or leukapheresis product
The P140K-MGMT vector is manufactured cGMP grade and has been used in previous trials
Media and components for cell culture and transduction are all pharmaceutical cGMP grade, Zoledronate (Novartis) and IL-2 (Miltenyi) cGMP grade and approved US & EU
Residual TCR-αβ T cells are removed by immunomagnetic purification in closed system (CliniMACS; Miltenyi Biotec)
The cell manufacturing protocol has been reviewed by FDA in a pre-IND teleconference
In Vitro Findings – Purity and Potency of Transduced Cells are no Different than Unmanipulated Cells
Drug Resistant Immunotherapy enhances the effect of TMZ against Resistant Tumors
Intellectual Property

- US Patent 7,078,034 *In vitro* activated gamma/delta T lymphocytes July 18, 2006
  - L. Lamb, Palmetto Health, Columbia, SC

- “Drug Resistant Immunotherapy for Treatment of a Cancer” Emory University and UAB
  - US and Europe
  - Filed October 29th, 2010
  - Claims address system and methods to generating drug-resistant cytotoxic immune cells and uses thereof
  - H.T. Spencer, L. Lamb, A. Dasgupta
Summary and Conclusions

- Administration of P140K-MGMT transduced $\gamma\delta$ T cells during a drug challenge enhances killing of Temozolomide-sensitive and Temozolomide-resistant tumors over either separate treatment.
- Technological hurdles of expanding and transducing therapeutic grade $\gamma\delta$ T cells have been overcome. The genetically engineered $\gamma\delta$ T cells retain their cytotoxic potential and exhibit powerful anti-tumor properties in the presence of drug.
- This approach can be used concurrently with other treatments such as mAbs, tumor vaccines, and other biologics.
- In addition to existing investigator-specific IP, DRI patents have been filed collaboratively by Emory and UAB.
Current Status

- We have received new grant and philanthropic funding to validate an animal model that will enable the testing of this therapy on several types of neural tumors.
- Final clinical validation of cell manufacturing is in progress. Quality and regulatory staff are in place as well as a full cGMP manufacturing facility.
- The investigator group has a wealth of experience in successful SBIR/STTR proposals, small biotechnology management, cell and gene therapy IDE/IND submission and advanced cell manufacturing.
DRI GANTT Chart

- DRI design and construction (discovery)
- Initial in vitro/in vivo characterization (proof of concept)
- Identification of manufacturing platform (UAB)
- Acquire project financing (NIH/Aflac/UAB)
- FDA pre-IND meeting (non modified gamma delta cells)
- Cell processing for preclinical studies
- Acquire IP
- cGMP production/CMC
- In vivo NSG studies
- Toxicology testing
- FDA pre-IND meeting (engineered cells)
- IND submission/review
- Human phase 1 clinical trial

Timeline:

- Jan 2009
- Apr 2010
- Jul 2011
- Oct 2012
- Jan 2013
- Apr 2014

www.braintumor.org
Appendix Slides
MGMT and Temozolomide

- Temozolomide works by alkylating three specific residues on replicating DNA – the O$_6$ position of guanine, the N$_3$ position of adenine and the N$_7$ position of guanine.
- When the O$_6$ position of guanine is methylated, DNA replication cannot take place, the DNA strand breaks, and eventually the cell dies.
- This process can be overcome by the enzyme methylguanine methyltransferase – MGMT – which removes the methyl group from the O$_6$ position and allows normal DNA repair to take place.
The concept of drug resistance immunotherapy (DRI)

Treatment Paradigm

Chemotherapy blunts both a natural and therapeutic anti-tumor immunity

Immune protection retains anti-tumor immunity during maximum vulnerability of the tumor
Chemistry (cont.)

- **Product release:**
  - Purity: $\gamma\delta$ T cells $>60\%$, secondary component NK cells, $\alpha\beta$ T cells $<1\%$
  - Potency: $>50\%$ lysis of U251MG at E:T 20:1 in 4-hour cytotoxicity assay
  - Toxicology: $<5\%$ lysis of cultured human astrocytes at E:T 20:1 in 4h
  - Sterility: negative gram stain and rapid endotoxin (Immediate), negative 14 day cultures for aerobic, anaerobic, and mycoplasma.

**Proliferation of Non-Modified vs. Transduced $\gamma\delta$ T cells in Culture**

<table>
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<tr>
<th>Specimen</th>
<th>Initial $\gamma\delta$ T cell number</th>
<th>Final* (unmodified)</th>
<th>Fold Expansion</th>
<th>Final* (transduced)</th>
<th>Fold Expansion</th>
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<td>20100504</td>
<td>$5.1 \times 10^6$</td>
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<td>20110308</td>
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<td>$1.2 \times 10^9$</td>
<td>438.5</td>
<td>$5.4 \times 10^8$</td>
<td>191.4</td>
</tr>
</tbody>
</table>
Pharmacology (Vector) – Genetic Engineering of $\gamma\delta$ T cells

SIV(bottom) and HIV (top) Effectively transduce $\gamma\delta$ T cells

Genetic modification of $\gamma\delta$ T cells with MGMT is protective against TMZ toxicity

With 200 $\mu$M TMZ

MOI = 25

SIV

HIV

Viable Cells/$\mu$l

Copies per cell

MOI

Temozolomide ($\mu$M)

Visibilities TMZ-treated $\gamma\delta$ cells

Topro APC-A

www.braintumor.org

National Brain Tumor Society
DRI Immunotherapy (drug resistant NK92 cells) + Chemotherapy

Non-DRI Immunotherapy (naïve NK92 cells) + Chemotherapy

Chemotherapy Only

Immunotherapy Only (drug resistant NK92 cells)

Control Untreated Tumor

IMR5 cells implanted s.c. 10-11 days

Start of Treatments

Timeline of Treatments

Week 1
Day 1 Therapy
Day 3 Therapy
Day 5 Therapy

Week 2
Day 8 Therapy
Day 10 Therapy

Week 3
Day 15 Therapy

CBC

www.brainmalignancies.org
High-Dose DRI Regimen: dose responsive

NK-92 cells, $3 \times 10^6$ + TMZ, 125 mg/kg

- Non-modified NK-92 + TMZ
- Nontreated
- Low Dose DRI
- High Dose DRI

Percent of Survival

Days after challenge

Tumor size (sq. mm)

Non-modified NK-92

Drug resistant NK-92

Low Dose DRI

High Dose DRI

Non-modified NK-92+TMZ

Percent of Survival

Days after challenge
IND-Enabling Animal Testing

Primary endpoints: Survival
Tumor size
Hematopoietic toxicity
Invasion of tumors by γδ T cells
Response of γδ T cells to early Syngeneic GL261 Mouse Glioma (Immunocompetent Model).