A multi-center clinical consortium to investigate the biology and clinical efficacy of autologous T cells genetically targeted to the CD19 antigen in patients with B cell malignancies

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The Approach

Utilization of a patient’s own T-cells, genetically modified in the laboratory to recognize their tumor cells as a novel approach to treat patients with B cell malignancies
Generation of an antigen specific chimeric antigen receptor (CAR)

Murine mAb

TCR complex

scFv-CD8-ζ

CAR retroviral vector
CD19

- CD19 expression is restricted to B cells and possibly follicular dendritic cells
- CD19 is not expressed on pluripotent bone marrow stem cells
- CD19 is expressed on the surface of most B cell malignancies
- Antibodies against CD19 inhibit growth of tumor cells

Diagram:
- Stem Cell
- pro B
- pre B
- immature B
- mature B
- plasma cell
- preB-ALL
- B cell lymphomas and leukemias
- myelomas
- CD19
- CD22
- CD20
Generation of CD19-targeted T cells for treatment of B cell malignancies

1. Construct a chimeric antigen receptor (CAR)

2. Subclone CAR gene into a retroviral vector (SFG)

3. Transduce and expand patient T cells \textit{ex vivo}

4. Infuse transduced T cells to eradicate CD19$^+$ tumor
Clinical trial overview

Autologous Blood Collection

Infusion of T Cells

Antibody-Coated Beads

Cells

Activation Expansion gene transfer

Bead Removal and Formulation

Product
The Problem

- There are many current ongoing phase I clinical trials targeting CD19 with many clinically relevant variables:
  - CAR gene transfer
    - Retrovirus
    - Lentivirus
    - Transposons
    - Electroporation
  - Targeted disease
    - Leukemia (CLL AML)
    - Lymphoma (NHL)
  - Patient population
    - Pediatric
    - Adult
  - Prior lymphodepletion (+/-)
  - CAR design
    - First generation CARs
    - Second and third generation CARs
Variables in CAR design

First-Generation CAR: scFv-CD3ζ
Second-Generation CAR: scFv-CD28-CD3ζ
Third-Generation CAR: scFv-CD28-4-1BB-CD3ζ, scFv-CD28-OX40-CD3ζ
The Result

<table>
<thead>
<tr>
<th>Center</th>
<th>Disease</th>
<th>CAR Endodomain</th>
<th>Vector to Express CAR</th>
<th>Conditioning Regimen</th>
<th>Target</th>
<th>Status</th>
<th>ClinicalTrials.Gov</th>
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</thead>
<tbody>
<tr>
<td>MSKCC</td>
<td>CLL –refractory</td>
<td>Zeta/28</td>
<td>RV</td>
<td>Cyclophosphamide</td>
<td>CD3-selected</td>
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<tr>
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<td>Zeta/28</td>
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<td>B NHL and CLL</td>
<td>Zeta/28 vs. Zeta</td>
<td>RV</td>
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<td>PBMC (OKT3 and IL-2)</td>
<td>Open. 5 treated: 4 DLBCL 1 B-CLL</td>
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<td>BCM</td>
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<td>Zeta/28 vs. Zeta-EBV</td>
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<td>BCM</td>
<td>B ALL, S/P HSCT</td>
<td>Zeta/28</td>
<td>RV</td>
<td>+30 days after allo-HSCT</td>
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<td>NCI</td>
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<td>Zeta/28</td>
<td>RV</td>
<td>Fludarabine and Cyclophosphamide</td>
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<td>U Penn</td>
<td>Refractory B Leukemia/Lymphoma</td>
<td>Zeta/41BB vs. Zeta-EBV</td>
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<td>Auto PBMC (CD3/CD28 beads)</td>
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<tr>
<td>U Penn</td>
<td>Relapsed ALL, S/P HSCT</td>
<td>Zeta/41BB</td>
<td>LV</td>
<td>Variable</td>
<td>Allo DLI</td>
<td>To open</td>
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<td>MDACC</td>
<td>B-NHL, S/P autologous HSCT</td>
<td>Zeta/CD28</td>
<td>Electroporation/ SB plasmids</td>
<td>BEAM-R</td>
<td>Auto PBMC (+/- IL-2)</td>
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<td>COH</td>
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<td>LV</td>
<td>Tcm: CD8+/CD4-/-CD45RA-/CD62+</td>
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Kohn et al Mol Ther (in press)
Currently open clinical trials of CD-19 targeted T cells: MSKCC, UPenn, and CHOP

MSKCC Clinical Trials:
IRB 06-138: A Phase I Trial for the Treatment of Purine Analog-Refractory Chronic Lymphocytic Leukemia using Autologous T cells Genetically Targeted to the B cell Specific Antigen CD19
    Adult patients with CLL, 1928z retroviral vector, prior lymphodepletion (8 enrolled, 7 treated)

IRB 09-114: A Phase I Trial of B Cell Acute Lymphoblastic Leukemia (B-ALL) Treated with Autologous T cells Gene Targeted to the to the B cell Antigen CD19.
    Adult patients with ALL, 19-28z retroviral vector, prior lymphodepletion (3 enrolled, 1 treated)

UPenn Clinical Trial:
UPCC 04409: Pilot Study of Redirected Autologous T Cells Engineered To Contain Anti-CD19 Attached To TCR And 4-1BB Signaling Domains in Patients with Chemotherapy Resistant or Refractory CD19+ Leukemia and Lymphoma.
    Adult patients with refractory leukemia and lymphoma, 19-41BB lentiviral vector, prior lymphodepletion (3 enrolled, 3 treated)

CHOP Clinical Trial:
CHP-959: Phase 2A Study of Redirected Autologous T cells Engineered to Contain anti-CD19 attached to TCR and Co-stimulatory Signaling Domains in Patients with Chemotherapy Resistant or Refractory CD19+ Leukemia and Lymphoma.
    Pediatric patients with refractory leukemia and lymphoma, 19-41BB lentiviral vector, prior lymphodepletion
A Solution (with Multiple Endpoints)

To generate a multi-center consortium utilizing harmonized pre-existing ongoing clinical trials to address variables in gene transfer and CAR design, as well as validate this therapeutic approach in larger multi-center clinical trials. To directly compare retroviral and lentiviral vectors for gene transfer of CARs into patient T cells.

1) To directly compare *in vivo* persistence and anti-tumor efficacy of second generation CD19 targeted CARs containing either the CD28 or 4-1BB co-stimulatory domains.

2) To more rapidly generate statistically relevant data regarding these variables.

3) To further demonstrate the “exportability” of this technology between academic institutions suitably endowed with requisite GMP facilities validating the feasibility of conducting future planned phase II-III clinical trials utilizing this technology.
PLAN: Harmonizing currently open clinical trials of CD-19 targeted T cells at MSKCC, UPenn, and CHOP

1) Protocols for both retroviral and lentiviral gene transfer will be harmonized between MSKCC, UPenn, and CHOP.

2) UPenn will provide MSKCC with 19-41BBz lentiviral vector (BB-IND 13960) under cross reference to validate gene transfer in the clinical setting at MSKCC, while clinical grade retroviral 19-28z vector from MSKCC (BB-IND 13266) will be provided to UPenn for similar validation.

3) All clinical trials at MSKCC, UPenn, and CHOP will be modified to adhere to the planned harmonized protocol of treating all subsequent patients on these protocols with equal numbers of retrovirally transduced 19-28z T cells and lentivirally transduced 19-41BBz T cells, the sum of infused T cells being equal to the total of planned infused modified T cells as stipulated in the respective initial clinical trials.

4) Immune monitoring of modified T cells and endogenous immune effectors will be conducted in part through collaborative efforts by the immune monitoring cores at each center (Kalos UPenn, and Jianda MSKCC) (Aim 2).

5) Ideally, collated analyses of vector and CAR design in these proposed studies will overcome variables including the targeted B cell malignancy and patient selection (adult versus pediatric patients) providing data to support recommendation of the better viral vector construct to generate the optimal number of tumor targeted T cells as well as the superior CD19 targeted CAR construct to induce optimal in vivo anti-tumor responses and modified T cell persistence.
Meeting Criteria for IRM STRAP Funding: Development over Discovery

- **Is this a clinically significant translational cancer research opportunity that should be accelerated?**
  - Yes. Preclinical and clinical data generated at our institutions as well as others demonstrate that adoptive therapy with genetically targeted tumor specific T cells have significant clinical promise worthy of accelerated development.

- **Does the proposal address one of the four prioritized areas in the Notice?**
  - Yes. This proposal addresses both adoptive therapy and “T body” or CAR therapy.

- **Is this an opportunity that would be difficult to accomplish through other available funding approaches?**
  - Yes. Significant funding for clinical development in the form of clinical trials, especially in a multi-center format, is difficult to obtain through other currently available RFAs.

- **Does the research plan address the developmental requirements of an IRM STRAP with components such as a well-described plan, reasonable timeframe/milestones, defined collaboration responsibilities, etc.?**
  - Yes. The proposal is a first of its kind multi-institutional collaborative effort designed to accelerate clinical development of adoptive T cell therapy of cancer with a uniquely reasonable time frame to begin accrual and therapy.

- **Is there a reasonable likelihood that the proposed research can reach IND status and clinical testing?**
  - Yes. INDs are already in place, and initiation of planned clinical trials will only require cross reference of INDs and IRB and FDA approval of modified/harmonized pre-existing clinical trials.

- **Does the proposed Research Team have the appropriate expertise and experience to accomplish the IRM STRAP?**
  - Yes. Investigators at MSKCC, UPenn, and CHOP have previously demonstrated expertise the conduct of adoptive T cell therapy trials, as well all investigators have demonstrated prior proficiency in navigating clinical trials through both institutional committees as well as federal agencies.
Milestones and (modified) Timeline

• **6-9 months:** Obtain IRB approvals for harmonized/modified clinical trials and FDA approval for cross-referencing of existing INDs.

• **6-12 months:** Initiate enrollment on modified protocols.

• **12-24 months:** Completion of preclinical comparative CAR and vector studies as described in Aim 3.

• **24-30 months:** Complete enrollment of 24 patients on collective clinical trials.

• **24-36 months:** Complete analyses of clinical and correlative data (Aim 2) from enrolled and treated patients on the collective clinical trials.
Progress to Date: Harmonizing Gene Transfer Protocols

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<th>CHOP</th>
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<td>Validation of gene transfer protocols</td>
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Progress to Date: Harmonizing Clinical Trials

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<td>IND cross reference and FDA submission</td>
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<td>Pend</td>
<td>Pend</td>
</tr>
<tr>
<td>Initiate enrollment of patients on modified trials</td>
<td>Pend</td>
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Relevance of IRM STRAP Award to Parent RO1 Award

- NIH-R01 Award (CA138738-01): Adoptive Immunotherapy of Cancer with IL-12 Secreting Tumor-Targeted T cells
  - The aims of this application are to investigate the enhanced in vivo anti-tumor potential of CD19 targeted T cells further modified to secrete the IL-12 cytokine.
  - The ultimate goal of this work is to translate this approach to the clinical setting.
  - Accelerated enrolment and analyses of patients treated on the “first generation” clinical protocols presented in this STRAP award will more rapidly provide much needed insight into the clinical in vivo biology and limitations of CAR modified T cells.
  - As well, the studies funded by the STRAP award, will likely therefore provide the rationale for “second generation” trials utilizing IL-12 secreting, CD19 targeted T cells with predicted enhanced anti-tumor efficacy and persistence.
Acknowledgements

• MSKCC
  – Renier Brentjens
  – Michel Sadelain
  – Isabelle Riviere
  – Marco Davila
  – Jae Park
  – Xiuyan Wang
  – Jolanta Stefanski
  – Clare Taylor
  – Raymond Yeh
  – Kevin Curran
  – MSKCC GTF Staff

• UPenn
  – Carl June
  – Gwen Binder
  – Michael Milone
  – David Porter
  – Anne Chew
  – Zoe Zheng
  – Bruce Levine
  – Adam Bagg
  – Michael Kalos
  – UPenn GTF Staff

• CHOP
  – Stephan Grupp
  – David Barrett
  – David Teachey
  – Jon Fish
  – Junior Hall
Multicenter Clinical CAR Consortium
shared technologies: CAR clinical trials, GMP cell manufacture

MSKCC
- CAR
  - CD19:CD28z Protocol
  - Adults
- Clinical Vector
  - MMLV retroviral Tools/Models
  - SCID/beige xenograft
dual imaging technology
  - (click beetle and Gaussian)

Penn
- CAR
  - CD19:41BBz Protocol
  - Adults
- Clinical Vector
  - SIN Lentiviral Tools/Models
  - aAPC technology

CHOP
- CAR
  - CD19:41BBz Protocol
  - Pediatric
- Clinical Vector
  - Tools/Models
  - pre-B ALL disseminated NSG xenograft
  - Ramos xenograft