A PHASE I STUDY EVALUATING THE USE OF ALLODEPLETED T CELLS TRANSDUCED WITH INDUCIBLE CASPASE 9 SUICIDE GENE AFTER HAPLOIDENTICAL STEM CELL TRANSPLANTATION

Principal Investigators
Malcolm K. Brenner, MB, PhD, FRCP, MRCPath.
Helen Heslop, MD, FRACP, FRCPA

Co-Investigators
Robert Krance, MD
George Carrum, MD
Catherine Bollard MD
Stephen Gottschalk MD
Kathryn Leung MD
G Douglas Myers MD
Rammurti Kamble MD
Nabil Ahmed MD
Alana Kennedy-Nasser MD
Jessica Shafer MD
Adrian Gee M.I. Biol, PhD
Cliona Rooney, PhD
Gianpietro Dotti MD
Yuriko Fujita MD
Ellen S. Vitetta, Ph.D.
John Schindler, Ph.D.

Statistician
Heidi Weiss, PhD

Texas Children’s Hospital
6621 Fannin Street
Houston, Texas 77030-2399

The Methodist Hospital
6565 Fannin Street
Houston, Texas 77030-2399

Collaborating with
University of Texas Southwestern Medical Center
5323 Harry Hines Blvd.
Dallas, Texas 75230-8576
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**IMPORTANT TELEPHONE NUMBERS:**

**Inclusion / Coordination / Follow-up / Technical questions:**

- Yu-Feng Lin (Research Coordinator/ Cell and Gene Therapy):
  tel. : 832-824-4258
  pager: 713-605 8989 ext 5284
  E-mail: yxlin@TexasChildrensHospital.org

**Eligibility check / Adverse effects report:**

- Malcolm Brenner, MB, PhD (Center for Cell and Gene Therapy):
  tel. : 832-824-4663
  E-mail : mbrenner@bcm.tmc.edu

- Helen Heslop, MD, PhD (Adult Bone Marrow Transplantation Program):
  tel. : 832-824-4662
  E-mail : heheslop@txccc.org

**Protocol Issues:**

- Bambi Grilley, RPh, CCRC, CCRA
  Protocol Director, Center for Cell and Gene Therapy
  Tel. : 832-824-3893 Email : bjgrille@txccc.org

**Statistical Issues:**

- Heidi Weiss, PhD
  Assistant Professor
  Tel: 713-798-1600 Email: hweiss@bcm.tmc.edu
Checklist for Patient Eligibility Prior to Enrolling on Study.

PATIENT ID:_____________      PATIENT NAME:_________________

AT TIME OF TRANSPLANT

All of the following questions must be answered YES for patient to be eligible:

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Patient with one of the following</td>
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<tr>
<td></td>
<td></td>
<td>ALL or high grade NHL stage III or IV after first relapse or with primary refractory disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Myelodysplastic syndrome</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AML after first relapse or with primary refractory disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CML</td>
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<tr>
<td></td>
<td></td>
<td>Hemophagocytic lymphohistiocytosis (HLH), familial hemophagocytic lymphohistiocytosis (FLH), viral-associated hemophagocytic syndrome (VAHS), patients with Severe Chronic active Epstein Barr virus infection (SCAEBV) with predilection for T or NK cell malignancy, X-linked lymphoproliferative disease (XLP)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lack of suitable conventional donor (i.e. 5/6 or 6/6 related or 5/6 or 6/6 unrelated donor) or presence of a rapidly progressive disease not permitting time to identify an unrelated donor</td>
</tr>
</tbody>
</table>

AT TIME OF INFUSION OF ALLODEPLETED CELLS

The following questions must be answered “Yes” for the patient to be eligible.

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
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<tbody>
<tr>
<td></td>
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</table>

Note: Patients who would be excluded from the protocol strictly for laboratory abnormalities can be included at the investigators discretion after approval by the CCGT Protocol Review Committee and the FDA reviewer

To register a patient, please contact the Bone Marrow Transplant/CCGT research coordinator at 832-824-4258. To check eligibility, call Dr Brenner (832) 824-4663, Dr Krance (823) 824-4661 or Dr Heslop (832) 824-4662.

____________________   __________
Signature of MD     Date
1. Objectives

1.1. Primary objectives

1.1.1. To determine the number of suicide gene-modified allodepleted donor lymphocytes that can be given to recipients of haploidentical stem cell transplants that will result in a rate of Grade III/IV GVHD of ≤ 25%.

1.1.2. To evaluate the biological and clinical effects of administration of AP1903, a dimerizer used to activate the suicide gene mechanism, in patients who develop Grade II GVHD.

1.2. Secondary objectives

1.2.1. To analyze the contribution of the gene-modified cells to immune reconstitution in these patients by measuring their survival, persistence and expansion.

1.2.2. To measure the overall and disease free survival, at 100 days and at 1 year.

2. Introduction, Background and Rationale

2.1. Haploidentical stem cell transplantation

While stem cell transplantation has proven an effective means of treating a wide variety of diseases involving hematopoietic stem cells and their progeny, a shortage of histocompatible donors has proved a major impediment to the widest application of the approach. The introduction of large panels of unrelated stem cell donors and or cord blood banks has helped to alleviate the problem, but many patients remain unsuited to either source. Even when a matched donor can be found, the elapsed time between commencing the search and collecting the stem cells usually exceeds three months, a delay that may doom many of the most needy patients.\(^1\) Hence there has been considerable interest in making use of HLA haploidentical family donors.\(^2,3\) Such donors may be parents, siblings or second-degree relatives. The problem of graft rejection may be overcome by a combination of appropriate conditioning and large doses of stem cells, while GVHD may be prevented by extensive T cell-depletion of the donor graft. The immediate outcomes of such procedures have been gratifying, with engraftment rate > 90% and a severe GVHD rate of < 10% for both adults and children\(^4,5\) even in the absence of post transplant immunosuppression. Unfortunately the profound immunosuppression of the grafting procedure, coupled with the extensive T cell-depletion and HLA mismatching between donor and recipient lead to an extremely high rate of post-transplant infectious complications, and contributed to high incidence of disease relapse.

2.2. Immune reconstitution by addback of selectively allodepleted T cells

Donor T cell infusion is an effective strategy for conferring anti-viral and anti-tumor immunity following allogeneic stem cell transplantation.\(^6-8\) Simple addback of T cells to the patients after haploidentical transplantation, however, cannot work; the frequency of alloreactive T cells is several orders of magnitude higher than the frequency of, for example, virus specific T lymphocytes. We, and others, have been studying means to accelerate immune reconstitution by administrating donor T cells that have first been depleted of alloreactive cells.\(^9-11\) We achieve this by stimulating donor T cells with recipient EBV-transformed B lymphoblastoid cell lines (LCLs).\(^12\) Alloreactive T cells
upregulate CD25 expression, and are eliminated by a CD25 Mab immunotoxin conjugate, RFT5-SMPT-dgA. This compound consists of a murine IgG1 anti-CD25 (IL-2 receptor alpha chain) conjugated via a hetero-bifunctional crosslinker [N-succinimidyloxycarbonyl-alpha-methyl-d- (2-pyridylthio) toluene] to chemically deglycosylated ricin A chain (dgA).\textsuperscript{13}

We have used LCL as stimulators because of the following advantages: (1) They are excellent antigen-presenting cells  (2) They are readily prepared in large number even in heavily pre-treated patients (3) They lack T cell and myeloid lineage restricted minor histocompatibility antigens that may be targets for graft versus leukemia effect (4) They will be free of contamination with malignant cells and will therefore not present any tumor specific antigens.

Treatment with CD25 immunotoxin after LCL stimulation depletes >90% of alloreactive cells.\textsuperscript{12} In a phase I clinical study, using CD25 immunotoxin to deplete alloreactive lymphocytes we compared immune reconstitution after allodepleted donor T cells were infused at 2 dose levels into recipients of T-cell-depleted haploidentical SCT. Eight patients were treated at $10^4$ cells/kg/dose, and 8 patients received $10^5$ cells/kg/dose. Patients receiving $10^5$ cells/kg/dose showed significantly improved T-cell recovery at 3, 4, and 5 months after SCT compared with those receiving $10^4$ cells/kg/dose ($P < .05$).\textsuperscript{11} Accelerated T-cell recovery occurred as a result of expansion of the effector memory (CD45RA\textsuperscript{-}CCR\textsuperscript{-}) population ($P < .05$), suggesting that protective T-cell responses are likely to be long lived. T-cell-receptor signal joint excision circles (TRECs) were not detected in reconstituting T cells in dose-level 2 patients, indicating they are likely to be derived from the infused allodepleted cells.\textsuperscript{11} Spectratyping of the T cells at 4 months demonstrated a polyclonal Vbeta repertoire. Using tetramer and enzyme-linked immunospot (ELISPOT) assays, we have observed cytomegalovirus (CMV)- and Epstein-Barr virus (EBV)-specific responses in 4 of 6 evaluable patients at dose level 2 as early as 2 to 4 months after transplantation, whereas such responses were not observed until 6 to 12 months in dose-level 1 patients. The incidence of significant acute (2 of 16) and chronic graft-versus-host disease (GVHD; 2 of 15) was low.\textsuperscript{11} These data demonstrate that allodepleted donor T cells can be safely used to improve T-cell recovery after haploidentical SCT. We subsequently escalated to $10^6$ cells/kg without evidence of GVHD.

Although this approach reconstituted antiviral immunity, relapse remained a major problem and 6 patients transplanted for high risk leukemia relapsed and died of disease. Higher T cell doses are therefore required to reconstitute anti-tumor immunity and to provide the hoped-for anti-tumor effect, since the estimated frequency of tumor-reactive precursors is 1 to 2 logs less than frequency of viral-reactive precursors.\textsuperscript{14-16} It is almost inevitable that in some patients, these doses of cells will be sufficient to trigger GvHD even after allodepletion. We therefore propose to increase the margin-of-safety of our approach by incorporating a suicide gene, inducible caspase 9 (iCasp9), in the allodepleted T cells, permitting their destruction should administration have adverse effects.

2.3. Suicide gene strategy as a safety switch in GVHD
As we reach doses required to restore full immunocompetence, there is a risk that we will encounter GVHD with increasing frequency. Hence, we will only be able to give a T cell dose that is effective in the majority of patients if we can minimize the risk of uncontrolled GVHD for the minority. One strategy for improving the safety of donor T cells is to transduce them with a suicide gene which will enable in vivo T cell destruction should GVHD occur. Proof of concept has been demonstrated in phase I and II studies in which Herpes Simplex Virus thymidine kinase (HSVtk) expressing donor T cells can be eliminated in allograft recipients following administration of ganciclovir, which is phosphorylated to its active form by the HSVtk transgene.\textsuperscript{17,18}

HSVtk has a number of drawbacks, the most important of which is immunogenicity: as a foreign protein, HSVtk is a target for CD4 and CD8 T cell-mediated immune response, which results in premature elimination of HSVtk-modified cells.\textsuperscript{19} Other drawbacks of HSVtk include restriction of killing to dividing cells, the unintended elimination of gene-modified cells when ganciclovir is used for treatment of CMV reactivation, and reports of ganciclovir resistance resulting from truncated HSVtk formed from cryptic splice donor and acceptor sites.

### 2.4. Inducible caspase 9 (iCasp9) suicide gene

Because of the drawbacks of HSVtk, there is a need to develop and validate alternative suicide genes. We investigated the suitability of an alternative suicide gene, inducible caspase 9 (iCasp9).\textsuperscript{20} iCasp9-mediated suicide is based on conditional dimerization of pro-apoptotic molecules, that are constructed from human proteins and therefore less likely to be immunogenic.\textsuperscript{20} iCasp9 is generated by joining a drug-binding domain to human caspase 9.\textsuperscript{20} The drug-binding domain consists of human FK506-binding protein (FKBP12) with an F36V mutation. This point mutation increases the binding affinity of FKBP12 to non-toxic synthetic homodimerizers, AP20187 or AP1903.\textsuperscript{21} Administration of AP20187 or AP1903 dimerizes and activates caspase 9; this activates downstream caspases, leading to apoptosis within 24 hours.

iCasp9 has numerous advantages over HSVtk. It is human-derived and hence less likely to be immunogenic. Furthermore, killing by iCasp9 is rapid and not restricted to dividing cells. Finally, iCasp9 suicide gene does not preclude the use of ganciclovir as prophylaxis or treatment of cytomegalovirus disease.

### 2.5. Safety of synthetic homodimerizer, AP1903

Patients who develop grade ≥II GVHD will be treated with 0.4mg/kg AP1903 as a 2-hour infusion. AP1903 has been evaluated as an Investigational New Drug (IND) by the FDA and has successfully completed a phase I clinical safety study.\textsuperscript{22} No significant adverse effects were noted when AP1903 was administered over a 0.01mg/kg to 1.0mg/kg dose range.\textsuperscript{22} The maximal plasma level attained over this dose range was 10 to 1275ng/ml AP1903 (equivalent to 7 to 892nM). In preclinical experiments we have shown that the iCasp9 suicide gene, functions efficiently (>90% apoptosis after treatment with dimerizer) and that down-modulation of transgene expression that occurred with time was rapidly reversed upon T cell activation, as would occur when alloreactive T cells encountered their targets.\textsuperscript{23}
2.6. CD19 as a surface selectable marker

The overall functionality of suicide genes depends on both the suicide gene itself and the marker used to select the transduced cells. To ensure that the majority of infused T cells carry the suicide gene, a selectable marker, truncated human CD19 (\(\beta\)CD19) and a commercial selection device, will be used to select the transduced cells to \(>90\%\) purity. We have demonstrated that \(\beta\)CD19 enrichment can be performed with high purity and yield and, more importantly, the selection process had no discernable effect on subsequent cell growth and functionality.\(^{23}\)

3. Generation of Cell Therapy Product

All of these procedures will be performed in our GMP facility as dictated by Standard Operating Protocols (SOP). Brief summaries are given here.

3.1 Source Material

Up to 240 ml (in 2 collections) of peripheral blood will be obtained from the transplant donor according to the procurement consent. In some cases depending on the size of donor and recipient, a leukopheresis will be performed to isolate sufficient T cells. 10-20cc blood will be obtained from the recipient to generate the Epstein Barr virus (EBV)-transformed lymphoblastoid cell line used as stimulator cells.

3.2 Generation of Allodepleted Cells

Allodepleted cells will be generated from the transplant donors as previously described\(^{11}\) in our GMP facility according to our SOPs. In brief, peripheral blood mononuclear cells (PBMCs) from healthy donors are co-cultured with irradiated recipient Epstein Barr virus (EBV)-transformed lymphoblastoid cell lines (LCL) at responder-to-stimulator ratio of 40:1 in serum-free medium (AIM V; Invitrogen, Carlsbad, CA). After 72 hours, activated T cells that express CD25 are depleted from the co-culture by overnight incubation in RFT5-SMPT-dgA immunotoxin.\(^{11}\) Allodepletion is considered adequate if the residual CD3\(^+\)CD25\(^+\) population was \(<1\%\) and residual proliferation by \(^3\)H-thymidine incorporation was \(<10\%\).\(^{11}\)

3.3 Retroviral Production

A retroviral producer line clone has been generated for the iCasp9-CD19 construct. A master cell-bank of the producer will be generated. The master-cell bank will be tested to exclude generation of replication competent retrovirus and infection by Mycoplasma, HIV, HBV, HCV and others. The producer line will be grown to confluency. Supernatant is then harvested, filtered, aliquoted and rapidly frozen and stored at \(-80^\circ\)C. All batches of retroviral supernatant will be tested again in particular to exclude Replication Competent Retrovirus (RCR) and issued with a certificate of analysis as directed by our SOPs.

3.4 Transduction of Allodepleted Cells

Allodepleted T-lymphocytes are transduced using Fibronectin. Plates or bags are coated with recombinant Fibronectin fragment CH-296 (RehornectinTM, Takara Shuzo, Otsu, Japan). Virus is attached to retronectin by incubating producer supernatant in coated plates or bags. Cells are then transferred to virus coated plates or bags. After transduction allodepleted T cells will be expanded feeding them with IL-2 twice a week to reach the sufficient number of cells as per protocol.

3.5 CD19 Immunomagnetic Selection
Immunomagnetic selection for CD19 will be performed 4 days after transduction. Cells are labeled with paramagnetic microbeads conjugated to monoclonal mouse anti-human CD19 antibodies (Miltenyi Biotech, Auburn, CA) and selected on a CliniMacs Plus automated selection device. CD19-selected cells are expanded for a further 4 days and cryopreserved on day 8 post transduction.

### 3.6 Freezing

Aliquots of cells will be removed for testing of transduction efficiency, identity, phenotype and microbiological culture as required for final release testing by the FDA. The cells will then be cryopreserved prior to administration according to SOPs.

### 4. Study Drugs

#### 4.1. RFT5-SMPT-dgA

RFT5-SMPT-dgA is a murine IgG1 anti-CD25 (IL-2 receptor alpha chain) conjugated via a hetero-bifunctional crosslinker [N-succinimidyloxycarbonyl-alpha-methyl-d- (2-pyridylthio) toluene] (SMPT) to chemically deglycosylated ricin A chain (dgA). RFT5-SMPT-dgA is formulated as a sterile solution at 0.5 mg/ml. Thus, a vial with 5 mls contains 2.5 mg immunotoxin. The product will be used ex-vivo in the BCM CCGT facility according to SOPs.

#### 4.2. Synthetic homodimerizer, AP1903

Patients who develop grade =II GVHD will be treated with 0.4mg/kg AP1903 as a 2-hour infusion. AP1903 has been evaluated as an Investigational New Drug (IND) by the FDA and has successfully completed a phase I clinical safety study. No significant adverse effects were noted when AP1903 was administered over a 0.01mg/kg to 1.0mg/kg dose range. Our patients will receive 0.4 mg/kg of AP1903 as a 2 h infusion – based on published Pk data which show plasma concentrations of 10-1275ng/mL over the 0.01mg/kg to 1.0mg/kg dose range with plasma levels falling to 18% and 7% of maximum at 0.5 and 2hrs post dose. Although our pre-clinical data suggests rapid apoptosis in response to a single brief exposure to the dimerizer, we will propose administering 2 additional doses every other day (total 3 doses) to ensure tissue penetration with adequate concentrations for a sustained period.

#### 4.3. SFG.iCasp9.2A.?CD19 Retroviral Vector

SFG.iCasp9.2A.?CD19 consists of inducible caspase 9 (iCasp9) linked, via a cleavable 2A-like sequence, to truncated human CD19 (?CD19) iCasp9 consists of a human FK506-binding protein (FKBP12; GenBank AH002 818) with an F36V mutation, connected via a Ser-Gly-Gly-Gly-Ser linker to human caspase 9 (CASP9; GenBank NM 001229). The F36V mutation increases the binding affinity of FKBP12 to the synthetic homodimerizer, AP20187 or AP1903. The caspase recruitment domain (CARD) has been deleted from the human caspase 9 sequence because its physiological function has been replaced by FKBP12, and its removal increases transgene expression and function. The 2A-like sequence encodes an 18 amino acid peptide from Thosea Asigna insect virus, which mediates >99% cleavage between a glycine and terminal proline residue, resulting in 17 extra amino acids in the C terminus of iCasp9, and one extra proline residue in the N terminus of CD19. ?CD19 consists of full length CD19 (GenBank NM 001770) truncated at amino acid 333 (TDPTTRRF), which shortens the intracytoplasmic domain from 242 to 19 amino acids, and removes all conserved tyrosine residues that are potential sites for phosphorylation.
4.4 CD19 Immunomagnetic Selection

Immunomagnetic selection for CD19 will be performed by labeling allodepleted cells with paramagnetic microbeads conjugated to monoclonal mouse anti-human CD19 antibodies (Miltenyi Biotech, Auburn, CA) and selecting on a CliniMacs Plus automated selection device.

5. Patient Eligibility

5.1 Inclusion Criteria at the time of transplant

5.1.1 Patients with:
5.1.1.1 ALL or high grade NHL that is Stage III or IV and has relapsed or is considered to be primary refractory disease.
5.1.1.2 Myelodysplastic syndrome.
5.1.1.3 AML after first relapse or with primary refractory disease.
5.1.1.4 CML
5.1.1.5 Hemophagocytic lymphohistiocytosis (HLH), familial hemophagocytic lymphohistiocytosis (FLH), viral-associated hemophagocytic syndrome (VAHS), patients with Severe chronic active Epstein Barr virus infection (SCAEBV) with predilection for T or NK cell malignancy, X-linked lymphoproliferative disease (XLP).

5.1.2 Lack of suitable conventional donor (i.e. 5/6 or 6/6 related or 5/6 or 6/6 unrelated donor) or presence of a rapidly progressive disease not permitting time to identify an unrelated donor.

5.2 Inclusion Criteria at time of allodepleted T cell infusion

5.2.1 GVHD < Grade 2
5.2.2 Engrafted with ANC >500. Must not have less than 50% donor chimerism in either peripheral blood or bone marrow or relapse of their original disease.
5.2.3 Life expectancy > 30 days
5.2.4 Lansky/Karnofsky scores > 60
5.2.5 No severe intercurrent infection
5.2.6 Absence of severe renal disease (Creatinine > 3X normal for age)
5.2.7 Absence of severe hepatic disease (direct bilirubin > 3mg/dl, or SGOT > 500
5.2.8 Patient/Guardian able to give informed consent.

Note: Patients who would be excluded from the protocol strictly for laboratory abnormalities can be included at the investigator’s discretion after approval by the CCGT Protocol Review Committee and the FDA reviewer.

5.3 To register a patient, please contact the CCGT research coordinator at (832) 824-4258. To check eligibility, call Dr Brenner (832) 824-4663, Dr Krance (832) 824-4661 or Dr Heslop (832) 824-4662

6. Treatment Plan

6.1 Transplant Recipients

Patients will be scheduled to receive a haploidentical stem cell transplant on our institutional protocols for haploidentical transplant which use CD34-selected mobilized peripheral blood as the source of stem cells for recipients of HLA haploidentical related
donor stem cell grafts. At the time of transplant evaluation, this ancillary study of
allodepleted T cells will be discussed with the recipient and donor. If they agree to
participate, the recipient will sign a procurement consent for collection of 10-30cc blood
to generate the lymphoblastoid cell line to be used as stimulator cells in the production
process.

6.2 Donor Selection:
These protocols are open to patients who lack a 5/6 or 6/6 HLA antigen matched donor.
For this protocol, the “best” donor will be defined as a first-degree haploidentical family
member who matches at the most MHC loci. Matching will be determined by class I and
class II DNA typing. The donor should be sufficiently healthy not to be at increased risk
from the mobilization procedure. Should more than one “equally” MHC incompatible
donor be identified, other selection criteria will include age and size of donor, CMV
status and sex. The Principal Investigator will make the final decisions.

6.3 Donor Collections:
Up to 240 ml (in 2 collections) of peripheral blood will be obtained from the transplant
donor according to the procurement consent. In some cases depending on the size of
donor and recipient, a leukopheresis will be performed to isolate sufficient T cells either
prior to  stem cell mobilization or seven days after the last dose of G-CSF.

6.4 Timeline for Generation of Product

<table>
<thead>
<tr>
<th>Time</th>
<th>Donor</th>
<th>Recipient</th>
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</thead>
<tbody>
<tr>
<td>Pre transplant</td>
<td>Identify BMT donor</td>
<td>Prepare recipient LCLs</td>
</tr>
<tr>
<td>When LCL line available</td>
<td>Obtain 240ml of blood or unstimulated</td>
<td></td>
</tr>
<tr>
<td></td>
<td>leukapheresis. Prepare T cells and culture</td>
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<tr>
<td></td>
<td>with recipient or appropriate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1st degree relative</td>
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<tr>
<td></td>
<td>LCLs; prepare donor</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LCLs for later immune</td>
<td></td>
</tr>
<tr>
<td></td>
<td>reconstitution studies</td>
<td></td>
</tr>
<tr>
<td>2 days later</td>
<td>Treat T cells with RFT5-dgA</td>
<td></td>
</tr>
<tr>
<td>3 days later</td>
<td>Transduce</td>
<td></td>
</tr>
<tr>
<td>4 days later</td>
<td>CD19 selection</td>
<td></td>
</tr>
<tr>
<td>4 days later</td>
<td>Check samples for sterility/endotoxin, and</td>
<td></td>
</tr>
<tr>
<td></td>
<td>allodepletion; freeze remainder</td>
<td></td>
</tr>
<tr>
<td>From 30 days post transplant</td>
<td>Thaw and infuse T cells 30 days post</td>
<td></td>
</tr>
</tbody>
</table>
6.5 Administration of Allodepleted T cells

6.5.1 Recipients who meet eligibility criteria will be eligible to receive allodepleted cells form thirty days following transplantation (day +30) if the product has completed final release testing and a certificate of analysis has been issued by Quality Assurance in the GMP facility. The cryopreserved T cells will be thawed and infused through a catheter line with normal saline. The patient will be premedicated with hydrocortisone 100 mg IV and Benadryl 25 mg IV. Outpatients may be treated in the clinic and monitoring will be undertaken according to institutional standards. All treatment will be given at CAGT in The Methodist Hospital or Texas Children’s Hospital.

6.5.2 This study will begin with a dose of T cells that did not cause GvHD in haploidentical recipients in our previous study and escalate in half log increments. A continual reassessment method based on a logistic dose-response curve with cohorts of size 2 will be employed to determine the MTD. Cohorts of size 2 will be accrued beginning at dose level 1 and the dose-response curve is estimated after toxicity outcome as defined in section 8.2 is observed to determine the recommended dose level for the next patient cohort. Each patient will receive up to five additional injections of T cells at the same dose, at monthly intervals, provided there is no evidence of grade 2 or higher GVHD, until total T cell numbers are > 1000/μl

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>Dose</th>
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<tbody>
<tr>
<td>1</td>
<td>1 x 10^6 T cells/Kg</td>
</tr>
<tr>
<td>2</td>
<td>3 x 10^6 T cells/Kg</td>
</tr>
<tr>
<td>3</td>
<td>1 x 10^7 T cells/Kg</td>
</tr>
</tbody>
</table>

6.5.3 Patients may be enrolled on the next dose level of T cells when all patients on the previous dose level have reached Day 40 days following initial - T cell infusion without unacceptable toxicity as defined in section 8.2 below.

6.6 Administration of AP1903 Dimerizer Drug

Patients who develop Grade II or greater signs of GvHD after infusion of allodepleted T cells will have a tissue biopsy where feasible to confirm the diagnosis and will then receive 0.4 mg/kg of AP1903 as a 2 h infusion – based on published Pk data which show plasma concentrations of 10-1275ng/mL over the 0.01mg/kg to 1.0mg/kg dose range with plasma levels falling to 18% and 7% of maximum at 0.5 and 2hrs post dose. Although our pre-clinical data suggests rapid apoptosis in response to a single brief exposure to the dimerizer, we will propose administering 2 additional doses every other day (total 3 doses) to ensure tissue penetration with adequate concentrations for a sustained period. If patients show progression of GvHD or fail to improve by at least 1 grade within 4 days, they will be placed on conventional GvHD therapy as per institutional SOPs.
7 Evaluation During Study

7.1 General evaluations will be conducted as per standard of care for patients after receiving a haploidentical PBSCT.

7.1.1 Donor Engraftment will be evaluated via standard studies on peripheral blood (FISH/DNA studies for chimerism).

7.1.2 CMV antigenemia monitoring; EBV PCR as per SOP.

7.1.3 Viral, protozoal, bacterial and fungal infections will be monitored according to our SOPs.

7.2 Study Specific Evaluations:

7.2.1 Physical examination with each visit.

7.2.2 Toxicity and GVHD grading with each visit.

7.2.3 The detailed schedule for laboratory monitoring of immune recovery is included below.

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wk 0 M1 M2 M3 M4 M5 M6 M7 M8 M9</td>
<td>M12 then annually X 15 years (total)</td>
</tr>
<tr>
<td>Infusion T cells</td>
<td>X X X X X</td>
<td></td>
</tr>
<tr>
<td>Hx</td>
<td>X X X X X X X X X X X X</td>
<td></td>
</tr>
<tr>
<td>PE</td>
<td>X X X X X X X X X X X X</td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>X X X X X X X X X X X X</td>
<td></td>
</tr>
<tr>
<td>VS</td>
<td>X X X X X X X X X X X X</td>
<td></td>
</tr>
<tr>
<td>Pulse Ox</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Performance Status</td>
<td>X X X X X X X X X X X X</td>
<td></td>
</tr>
<tr>
<td>Pregnancy Test</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>CBC d/p</td>
<td>X X X X X X X X X X X X</td>
<td></td>
</tr>
<tr>
<td>Lytes/BUN/Cr</td>
<td>X X X X X X X X X X X X</td>
<td></td>
</tr>
<tr>
<td>AST/Bili/Alb</td>
<td>X X X X X X X X X X X X</td>
<td></td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Function, Persistence Studies</td>
<td>X X X X X X X X X X X X</td>
<td></td>
</tr>
<tr>
<td>RCR PCR and archive samples</td>
<td>X X X X X X</td>
<td>X</td>
</tr>
<tr>
<td>HAMA/HARA</td>
<td>X X X X X</td>
<td>X</td>
</tr>
</tbody>
</table>

7.2.3.1 A serum sample of 3ml to test for HAMA will be taken prior to the first infusion and a serum sample of 3ml to test for HAMA/HARA will be taken a month after each infusion of allodepleted T cells
7.2.3.2 Immune reconstitution
Depending on availability of patient cells and reagents, immune reconstitution studies (Immunophenotyping, T and B cell function) will be obtained at serial intervals after infusion of allo-depleted T cells. Approximately 10-60 of patient blood will be taken, if feasible, monthly x 9 months, and then at 1 and 2 years. The amount of blood taken will be dependent on the size of the recipient and will not exceed 10cc/kg in total (allowing for blood taken for clinical care and study evaluation) at any one blood draw.

7.2.3.3 Persistence and safety of transduced allo-depleted T cells
The following analysis will be performed on peripheral blood to monitor function, persistence and safety of transduced T-cells at time-points indicated in the study calendar.
- Phenotype to detect the presence of transgenic cells
- Quantitative real-time PCR to detect retroviral integrants.
- RCR testing by PCR. RCR testing will be performed pre study, 3, 6, 12 months and yearly for a total of 15 years. Aliquot of cells and serums will also be archived for use in future studies for RCR as required by the FDA or RAC.
- PCR to detect retroviral integrant clonality and integrant locus if transgenes detected at >0.5%.
- If there is insufficient blood for all the tests listed above at any time point, RCR testing and Quantitative real-time PCR will be the first priorities.

Studies will be conducted depending on the availability of the patient and the ability to safely draw the amount of blood needed for the studies. The time points given are approximate as patients may not always be able to keep appointments. However, every effort will be made, to obtain studies on the above-mentioned schedule.

7.3.4 Patients receiving AP1903
In patients who develop GVHD of Grade 2 or greater and receive AP1903 additional blood samples of 10-30cc will be obtained to monitor the effects of AP1903 on transgene persistence as follows.

<table>
<thead>
<tr>
<th>Test</th>
<th>Day 0</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP1903 dose</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Function, Persistence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Studies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenotyping</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

In addition if recipients have biopsies of GVHD sites as part of diagnosis of clinical care samples will be obtained for analysis of transgene persistence by PCR.

8. Criteria for Study Evaluation

8.1 Hematopoietic engraftment. Engraftment will be recorded as the day of absolute neutrophil counts exceeded $0.5 \times 10^9/ml$ on the first of 3 days, and chimerism studies demonstrate donor hematopoiesis.

8.2 The primary endpoint for T cell dose evaluation is grade III/IV acute GVHD. It will be graded by the method of Przepiorka et al.\(^{26}\)

8.3 Mortality will be recorded as day of death.
8.4 Toxicity will be graded according to NCI Common Criteria Index version 2.0 at [http://ctep.cancer.gov](http://ctep.cancer.gov). Grade 2 infusion related reactions will be considered “severe”.

8.5 Relapse will be assessed from regular blood and marrow analyses (see section 15) and will be recorded by the day of detection.

8.6 Immune reconstitution
Depending on availability of patient cells and reagents, immune reconstitution studies (Immunophenotyping, T and B cell function) will be obtained at serial intervals after transplant:

9 Statistical Analysis and Stopping rules.
9.1 The primary objectives of this protocol are (i) to determine the number of donor lymphocytes that can be given to recipients of haploidentical stem cell transplants after depletion of recipient-reactive T lymphocytes by ex-vivo treatment with RFT5-dgA immunotoxin, and will result in a rate of acute Grade III/IV GVHD of ≤25%; (ii) to analyze immune reconstitution in these patients and (iii) to measure their overall and disease free survival, at 100 days and at 1 year.

The MTD is defined to be the dose which causes grade III/IV acute GVHD in at most 25% of eligible cases. This will be estimated based on a modified continual reassessment method (CRM) using a logistic model with a cohort of size 2 (25). Three dose groups are being evaluated namely, $1 \times 10^6$, $3 \times 10^6$, $1 \times 10^7$ with prior probabilities of toxicity estimated at 10%, 15%, and 30%, respectively. In this proposed CRM design, we employ modifications to the original CRM (25) by accruing more than one subject in each cohort, limiting dose escalation to no more than one dose level, and starting patient enrollment at the lowest dose level shown to be safe for non-transduced cells. Toxicity outcome in the lowest dose cohort will be used to update the dose-toxicity curve. The next patient cohort is assigned to the dose level with an associated probability of toxicity closest to the target probability of 25%. This process continues until at least 10 patients have been accrued into this dose-escalation study. Depending on patient availability, we may plan to enroll at most 18 patients into this Phase I trial or until 6 patients have been treated at the current MTD. The final MTD will be the dose with probability closest to the target toxicity rate at these termination points.

We performed simulations to determine the operating characteristics of the proposed design and compared this with a standard 3+3 dose-escalation design. Our proposed design allows us to obtain better estimates of the MTD based on a higher probability of declaring the appropriate dose level as the MTD, afforded smaller number of patients accrued at lower and likely ineffective dose levels, and maintained a lower average total number of patients required for the trial. We expect a shallow dose-toxicity curve over the range of doses we propose in our study and therefore feel comfortable with slightly more accelerated dose-escalations without comprising patient safety. In fact, our simulations indicate that the modified CRM design will not incur a larger average number of total toxicities as compared to the standard design (total toxicities equal to 1.9 and 2.1, respectively.).

Grade III/IV GVHD that occurs within 40 days after initial infusion will be factored into the CRM calculations to determine the recommended dose for the subsequent cohort. During the study, real-time monitoring of patient toxicity outcome will be performed in
order to implement estimation of the dose-toxicity curve and determine dose level for the next patient cohort using one of the pre-specified dose levels.

9.2 Descriptive statistics will be utilized to summarize GVHD rates and other measures of safety and toxicity.

We will analyze several parameters measuring immune reconstitution resulting from iCaspase transduced allogeneic T cells. These include repeated measurements of total lymphocyte counts, T and CD20 B cell numbers, and FACS analysis of T cell subsets (CD3, CD4, CD56, CD45RA, CD45RO, alpha/beta and gamma/delta T cell receptors). Each subject will be measured pre-infusion and at multiple time points post-infusion as indicated in section 7 above. Thus, we expect a data-dense study despite the small patient numbers in this Phase I trial. Descriptive summaries of these parameters in the overall patient group and by dose group as well as by time of measurement will be presented. Growth curves representing measurements over time within a patient will be generated to visualize general patterns of immune reconstitution. The proportion of iCasp9 positive cells will also be summarized at each time point. Pairwise comparisons of changes in these endpoints over time compared to pre-infusion will be implemented using paired t-tests or Wilcoxon signed-ranks test.

We will then employ longitudinal analysis of each repeatedly-measured immune reconstitution parameter using the random coefficients model. This will allow us to model patterns of immune reconstitution per patient while allowing for varying intercepts and slopes within a patient. We will also include dose level as an independent variable in the model to account for the different dose levels received by the patients. From this model, we will be able to test whether there is a significant improvement in immune function over time and estimate the magnitude of these improvement based on estimates of slopes and its standard error. We will also evaluate any indication of differences in rates of immune reconstitution across different dose levels of CTLs. The normal distribution with an identity link will be utilized in these models and implemented using SAS MIXED procedure. We will assess the normality assumption of the immune reconstitution parameters and perform transformations (e.g. log, square root), if necessary to achieve normality. These modeling strategies will be considered exploratory based on the limited patient numbers in this initial trial.

A similar strategy as described above will be employed to assess kinetics of T cell survival, expansion and persistence. In addition, we will obtain the ratio of the absolute T cell numbers with the number of marker gene positive cells and model this longitudinally over time. A positive estimate of the slope will indicate increasing contribution of T cells for immune recovery. Likewise, evaluation of virus-specific immunity of the iCasp9 T cells will be accomplished by analysis of number of T cells releasing IFN gamma based on ex-vivo stimulation virus-specific CTLs using longitudinal models. Separate models will be generated for analysis of EBV, CMV and adenovirus evaluations of immunity.

Finally, we will summarize overall and disease-free survival in the entire patient cohort using Kaplan-Meier product-limit method. Proportion of patients surviving and who are disease-free at 100 days and 1 year will be estimated from the Kaplan-Meier curves.
10 Off Study Criteria:

10.1 Any patient who develops irreversible or life threatening or non-hematologic Grade 3 or 4 toxicity considered to be primarily related to the T cell infusion will be removed from this protocol and will not be eligible for subsequent T cell infusions.

10.2 Any questions regarding patients on this study should be addressed to Dr. Malcolm Brenner (832-824-4663).

11 Data and Protocol Management

11.1 Protocol Compliance: Patients will be reviewed weekly by the study investigators who will score the patient for study endpoints.

11.2 Reporting of serious adverse events (SAEs):

11.2.1 Within one (1) working day, report by telephone to the Principal Investigator or research nurse SAEs as defined below:

- Associated with the use of the drug: (FDA defines drug as RFT5-dgA allodepleted T cells and CD34+ cells): There is a reasonable possibility that the experience may have been caused by the drug.
- Disability: A substantial disruption of a person’s ability to conduct normal (change from baseline at study entry) life functions.
- Life threatening adverse drug experience: Any adverse drug experience that places the subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred.
- Serious adverse drug experience: Any adverse drug experience occurring at any dose that results in any of the following outcomes:
  - Death (including all deaths within 30 days of the last administration of study drug).
  - A life-threatening adverse drug experience.
  - Inpatient hospitalization or prolongation of existing hospitalization.
  - A persistent or significant disability/incapacity.
  - A congenital anomaly/birth defect.
  - Any other medical event that, in appropriate medical judgment, may require medical or surgical intervention to prevent one of the outcomes listed above.
- Unexpected adverse drug experience: Any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure, or, not consistent with the risk information described in the general investigational plan (protocol).

11.2.2 All serious adverse events as outlined in section 12.1 should also be reported within three (3) working days, in writing, to the Principal Investigator. An appropriate adverse event form should be used.
11.2.3 The sponsor will be responsible for reporting all SAEs to the appropriate regulatory agencies (e.g. FDA/NIH as applicable) as per each agencies individual reporting requirements and within that agencies required timeframe.

11.2.4 Non-serious adverse events will be reported to the appropriate regulatory agencies (e.g. IRB/FDA/NIH as applicable) at the time of the annual reports if required by that agency.

11.3 Data on all adverse experiences/toxicities (excluding all grade 1 fever and hematologic toxicities), regardless of seriousness must be collected (for documentation purpose only) through day 28 after the last infusion of allo-depleted T cells. This includes a change in the severity of the event (both increase and decrease in symptoms).

11.4 Documentation and reporting of SAEs (excluding all grade 1 fever and hematologic toxicities) will occur through day 28 after the last infusion of allo-depleted T cells. Thereafter, documentation and reporting of SAEs will be done per routine follow-up and standard practice for bone marrow transplant. Relapse and survival data will be collected.
### APPENDIX I: GVHD STAGES AND GRADES

<table>
<thead>
<tr>
<th>For skin:</th>
<th>Stage</th>
<th>Skin Involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>greater than 0, less than 25%</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>greater than or equal to 25%, less than or equal to 50%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>greater than 50%</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>greater than 50% with blisters</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>For gut:</th>
<th>Stage</th>
<th>Stool Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>less than 7 cc/kg</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>greater than or equal to 7 cc/kg, less than 14 cc/kg</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>greater than or equal to 14 cc/kg, less than 21 cc/kg</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>greater than or equal to 21 cc/kg, less than 28 cc/kg</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>greater than or equal to 28 cc/kg</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>For liver:</th>
<th>Stage</th>
<th>Bilirubin (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>less than 2</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>greater than or equal to 2, less than 3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>greater than or equal to 3, less than 6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>greater than or equal to 6, less than 15</td>
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</tr>
<tr>
<td>4</td>
<td>greater than or equal to 15</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Overall:</th>
<th>Grade</th>
<th>Organ Stage</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>skin = 1 or 2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>skin = 3, or skin less than or equal to 3 and gut or liver equal to 1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>skin greater than or equal to 3 and gut or liver equal to 2 or 3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>skin, gut or liver equal to 4</td>
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</tr>
</tbody>
</table>
REFERENCES


