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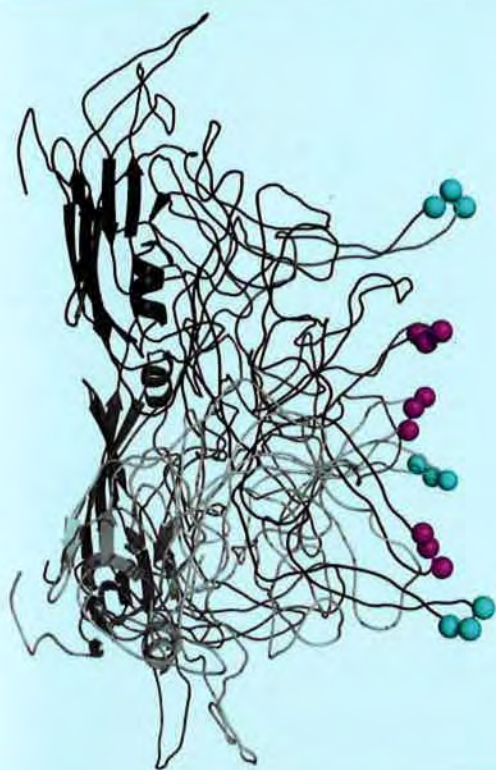


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Human Gene Therapy

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Human Gene Therapy

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EDITORIAL

A New Open Access Partner

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COMMENTARY

Double-Blinded, Placebo-Controlled, Randomized Gene Therapy Using Surgery for Vector Delivery

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RESEARCH ARTICLES

Infusing CD19-Directed T Cells to Augment Disease Control in Patients Undergoing Autologous Hematopoietic Stem-Cell Transplantation for Advanced B-Lymphoid Malignancies

P. Kebriaei, H. Huls, B. Jena, M. Munsell, R. Jackson, D.A. Lee, P.B. Hackett, G. Rondon, E. Shpall, R.E. Champlin, and L.J.N. Cooper

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Kebriaei and colleagues report on their phase 1 protocol to examine the safety and feasibility of administering genetically modified autologous T cells expressing a CD19-specific chimeric antigen receptor (CAR) to patients with high-risk B-lymphoid malignancies undergoing autologous hematopoietic stem cell transplantation. CAR was introduced to T cells by nonviral gene transfer, using the *Sleeping Beauty* system and signals through chimeric CD28 and CD3- ζ .

AAVrh.10-Mediated Expression of an Anti-Cocaine Antibody Mediates Persistent Passive Immunization That Suppresses Cocaine-Induced Behavior

J.B. Rosenberg, M.J. Hicks, B.P. De, O. Pagovich, E. Frenk, K.D. Janda, S. Wee, G.F. Koob, N.R. Hackett, S.M. Kaminsky, S. Worgall, N. Tignor, J.G. Mezey, and R.G. Crystal

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Rosenberg and colleagues construct an AAV serotype rh.10 vector that encodes the heavy and light chains of the high-affinity anti-cocaine monoclonal antibody GNC92H2. They demonstrate that intravenous administration of this vector leads to high serum levels of cocaine-specific antibodies that block administered cocaine from access to the brain, prevent cocaine-induced hyperlocomotor activity, and lead to anticocaine passive immunity in mice.

Immunodominant Liver-Specific Expression Suppresses Transgene-Directed Immune Responses in Murine Pompe Disease

P. Zhang, B. Sun, T. Osada, R. Rodriguez, X.Y. Yang, X. Luo, A.R. Kemper, T.M. Clay, and D.D. Koebel

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Zhang and colleagues investigate the mechanism for immunomodulatory gene therapy by evaluating two AAV vectors encoding human acid α -glucosidase (hGAA), including both a tolerogenic vector containing the liver-specific promoter (LSP) and an immunogenic vector containing the ubiquitously active cytomegalovirus enhancer and chicken β -actin promoter (CB) regulatory cassette. The authors find that simultaneous administration of tolerogenic and immunogenic vectors induces immune tolerance in mice and leads to higher levels of liver transduction, in comparison with either vector alone.

Characterization of an HIV-Targeted Transcriptional Gene-Silencing RNA in Primary Cells

A.-M.W. Turner, A.M. Ackley, M.A. Matrone, and K.V. Morris

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Turner and colleagues demonstrate that transcriptional gene silencing (TGS) can reduce viral transcription in primary human CD4⁺ T cells, although increasing viral burden resulted in the loss of this antiviral effect. Studies into off-target effects additionally identified a novel potential interaction between the small nucleolar RNA pathway and the TGS-based antisense RNA, resulting in activation of p53.

(continued)

Retargeting Vesicular Stomatitis Virus Using Measles Virus Envelope Glycoproteins 484*C. Ayala-Breton, G.N. Barber, S.J. Russell, and K.-W. Peng*

Ayala-Breton and colleagues use the measles virus (MV) hemagglutinin (H) and fusion (F) envelope glycoproteins to redirect vesicular stomatitis virus (VSV) entry and infection specifically to tumor-associated receptors such as epidermal growth factor receptor, folate receptor, and prostate membrane-specific antigen. *In vivo* expression of the all retargeted VSV was restricted to receptor-positive human tumor xenografts.

Development and Validation of Novel AAV2 Random Libraries Displaying Peptides of Diverse Lengths and at Diverse Capsid Positions 492*M. Naumer, Y. Ying, S. Michelfelder, A. Reuter, M. Trepel, O.J. Müller, and J.A. Kleinschmidt*

Naumer and colleagues expand the combinatorial AAV2 display system with a panel of novel AAV libraries displaying peptides of 5, 7, 12, 19, or 26 amino acids in length at capsid position 588 or 7-mer peptides at position 453. Characterization of vector selectivity by transduction of nontarget cells and comparative gene transduction analysis using a panel of 44 human tumor cell lines revealed that insertion of different-length peptides allows targeting of distinct cellular receptors for cell entry with similar efficiency, but different selectivity.

Silencing of miR20a Is Crucial for Ngn1-Mediated Neuroprotection in Injured Spinal Cord 508*M.K. Jee, J.S. Jung, Y.B. Im, S.J. Jung, and S.K. Kang*

Jee and colleagues demonstrate that small interfering RNA (siRNA)-mediated inhibition of microRNA 20a (miR20a) leads to increased expression of a key neuronal transcription factor, neurogenin-1 (Ngn1). They show that siRNA-mediated knockdown of miR20a enhances neural cell survival and neurogenesis and leads to recovery of motor function in a mouse model of traumatic spinal cord injury.

Asymmetric siRNA: New Strategy to Improve Specificity and Reduce Off-Target Gene Expression 521*Z. Yuan, X. Wu, C. Liu, G. Xu, and Z. Wu*

In this study, Yuan and colleagues demonstrate that asymmetrically shortened siRNAs can silence their targets as efficiently as their traditional 19 + 2 counterparts *in vitro*. Moreover, the authors suggest that shortening siRNAs from their 3' end improves the loading specificity of the RNA-induced silencing complex and results in fewer off-target gene expression effects.

BRIEF REPORT**Hepatic Gene Transfer in Neonatal Mice by Adeno-Associated Virus Serotype 8 Vector** 533*L. Wang, H. Wang, P. Bell, D. McMenamin, and J.M. Wilson*

Wang and colleagues conduct a series of preclinical animal studies examining the kinetics of AAV gene transfer. They demonstrate that self-complementary AAV8 results in robust and efficient hepatic gene transfer in neonatal mice. Yet, this transduction quickly decreases over a few weeks because of vector dilution caused by rapid cell proliferation in the liver of growing young mice.

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UCL Institute of Child Health, London

ABSTRACTS AVAILABLE ONLINE ONLY

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Cover: Adeno-associated virus type 2 (AAV2) capsid protein structures showing two peptide insertion sites at the top of the spikes around the threefold axes, enabling the development of AAV2 random libraries displaying peptides at two different positions. Insertion sites are marked together with two neighboring amino acids in purple (arginine 588) and cyan (glycine 453) in the side and top view of a capsid protein trimer. For further details, see the article by Naumer *et al.* on page 492.

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Infusing CD19-Directed T Cells to Augment Disease Control in Patients Undergoing Autologous Hematopoietic Stem-Cell Transplantation for Advanced B-Lymphoid Malignancies

Partow Kebriaei,¹ Helen Huls,² Bipulendu Jena,² Mark Munsell,¹ Rineka Jackson,² Dean A. Lee,^{2,3} Perry B. Hackett,⁴ Gabriela Rondon,¹ Elizabeth Shpall,¹ Richard E. Champlin,¹ and Laurence J.N. Cooper^{2,3}

Abstract

Limited curative treatment options exist for patients with advanced B-lymphoid malignancies, and new therapeutic approaches are needed to augment the efficacy of hematopoietic stem-cell transplantation (HSCT). Cellular therapies, such as adoptive transfer of T cells that are being evaluated to target malignant disease, use mechanisms independent of chemo- and radiotherapy with nonoverlapping toxicities. Gene therapy is employed to generate tumor-specific T cells, as specificity can be redirected through enforced expression of a chimeric antigen receptor (CAR) to achieve antigen recognition based on the specificity of a monoclonal antibody. By combining cell and gene therapies, we have opened a new Phase I protocol at the MD Anderson Cancer Center (Houston, TX) to examine the safety and feasibility of administering autologous genetically modified T cells expressing a CD19-specific CAR (capable of signaling through chimeric CD28 and CD3- ζ) into patients with high-risk B-lymphoid malignancies undergoing autologous HSCT. The T cells are genetically modified by nonviral gene transfer of the *Sleeping Beauty* system and CAR⁺ T cells selectively propagated in a CAR-dependent manner on designer artificial antigen-presenting cells. The results of this study will lay the foundation for future protocols including CAR⁺ T-cell infusions derived from allogeneic sources.

Introduction

LIMITED TREATMENT OPTIONS exist for patients with B-lymphoid malignancies who relapse after autologous or allogeneic hematopoietic stem-cell transplantation (HSCT). Regarding non-Hodgkin lymphoma (NHL), 50% of patients who relapse after conventional chemotherapy may be salvaged by autologous HSCT. Among those who are transplanted, residual disease at the time of transplantation predicts an increased rate of relapse (Mills *et al.*, 1995; Caballero *et al.*, 2003). Allogeneic HSCT, which may deliver a potent graft-versus-tumor (GVT) effect mediated by donor-derived immunocompetent cells, can be potentially curative in 30–50% of patients (Ratanatharathorn *et al.*, 1994; Verdonck, 1999). However, because of the high treatment-related mortality (TRM) associated with allogeneic HSCT, the GVT effect is not always correlated with improved overall survival (Peniket *et al.*, 2003). Thus, novel therapeutic approaches are urgently needed for these patients.

T-cell therapy can specifically target disease, employing anti-tumor mechanisms that are nonoverlapping with the conditioning regimen used for HSCT. Therefore, investigators have developed adoptive immunotherapy as a strategy to augment the GVT effect. To overcome immune tolerance to tumor-associated antigens (TAAs) investigators have developed and implemented immunotherapies infusing T cells genetically modified to express a chimeric antigen receptor (CAR) (Paulos *et al.*, 2008; Jena *et al.*, 2010). CARs are typically engineered to provide the genetically modified T cell with the specificity of a murine monoclonal antibody and, on binding a cell surface TAA, can specifically activate the T cell for proliferation, cytokine production, and cytotoxicity (Eshhar *et al.*, 1993; Brentjens *et al.*, 2003; Cooper *et al.*, 2003; Park *et al.*, 2007; Brenner and Heslop, 2010; Porter *et al.*, 2011). CARs are “universal” in that they bind TAAs independent of major histocompatibility complex (MHC), and thus one receptor construct can be used to treat a population

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of patients with the same TAA⁺ tumors. Multiple CAR designs have emerged to dock with multiple TAAs and to provide a fully competent activation signal in desired T-cell subsets (Berry *et al.*, 2009; June *et al.*, 2009; Jena *et al.*, 2010). CAR⁺ T cells have been tested in multiple trials at multiple centers, as we have reviewed (Jena *et al.*, 2010).

Here we describe a new Phase I cell and gene immunotherapy trial administering autologous *ex vivo*-expanded genetically modified T cells expressing a CD19-specific CAR into patients with high-risk B-lymphoid malignancies undergoing autologous HSCT. The rationale for redirecting the specificity of T cells for CD19, using a CAR, is based on the following: (1) CD19 is a B-lineage cell surface TAA expressed on lymphoid malignancies; (2) CD19 is not expressed on hematopoietic stem cells (HSC) and (3) soluble CD19 is apparently not shed into the circulation (to act as a competitor for binding CAR to CD19 on tumor cells). Preliminary studies demonstrated the safety, feasibility, and efficacy of infusing CD19-specific CAR therapy for lymphoid malignancies (Kochenderfer *et al.*, 2010). Our results on infusing a first-generation CD19-specific CAR into patients with recurrent follicular lymphoma after a preparative regimen consisting of rituximab and fludarabine demonstrated limited persistence due to incomplete signaling through CD3- ζ and an immune response to immunogenic transgenes coexpressed with CAR in T cells (Jensen, 2007). Therefore, we developed a new platform for the genetic modification of T cells based on (1) a second-generation CAR that signals through both chimeric CD28 and CD3- ζ (Singh, 2008), (2) electrotransfer of the *Sleeping Beauty* (SB) system to improve integration efficiency of CAR transgene, and (3) outgrowth of CAR⁺ T cells on designer artificial antigen-presenting cells (aAPC) to select for T cells with proven proliferative potential.

Clinical Trial Design

Regulatory approvals

The clinical trial is open only at the MD Anderson Cancer Center (MDACC, Houston, TX) as IRB #2007-0635 (IBC #RM0508-070). Approval was obtained from the NIH Office of Biotechnology Activities (OBA, #0804-922) and U.S. Food and Drug Administration (FDA) (IND #14193). The trial is listed at ClinicalTrials.gov (identifier: NCT00968760).

Objectives

The primary objective is to assess the safety, feasibility, and persistence of autologous *ex vivo*-expanded genetically modified CD19-specific CAR⁺ T cells intravenously administered to patients with persistent or relapsed CD19⁺ lymphoid malignancies. The secondary objectives will include (1) screening for the development of host immune responses against the CD19-specific CAR, (2) describing the ability of the infused T cells to home to sites of disease, such as bone marrow and lymph nodes, (3) assessing the impact of interleukin (IL)-2 on T-cell persistence, and (4) assessing disease response.

Patient eligibility

Patients are eligible if they are between 18 and 60 years of age with advanced CD19⁺ lymphoid malignancies, including non-Hodgkin's lymphoma (NHL), small lymphocytic

lymphoma (SLL), chronic lymphocytic leukemia (CLL), follicular lymphoma, or mantle cell lymphoma; that are beyond first relapse or primary refractory to conventional treatment. Additional eligibility criteria include adequate organ function, a Zubrod performance status of 0 or 1, no evidence of uncontrolled infection, and negative serology for hepatitis B (HBV), hepatitis C (HCV), and HIV. Patients with active CNS disease are excluded. In addition, patients with known allergy to bovine or murine products will be excluded. Furthermore, patients must not be taking systemic corticosteroids within 3 days before T-cell infusion or experiencing any new significant toxicity within 24 hours before T-cell infusion. Last, to be eligible for IL-2 injections after administrations of T cells, patients must not experience any new significant adverse events (AEs) probably or definitely attributed to the adoptively transferred T cells.

T cell manufacture and release

The two SB DNA plasmids (manufactured by Waisman Biomanufacturing, Madison, WI), encoding the CAR (designated CD19RCD28) transposon (Cooper, 2007; Singh *et al.*, 2008; Jena *et al.*, 2010) and SB11 transposase (Singh *et al.*, 2008; Manuri *et al.*, 2009), will be simultaneously electrotransferred into T cells with Nucleofector device (Lonza Group, Basel, Switzerland). The genetically modified T cells will be selectively propagated in a CAR-dependent manner on γ -irradiated K562 cells that have been genetically modified by transduction with lentivirus (in collaboration with C. June at the University of Pennsylvania, Philadelphia, PA) and cloned by limiting dilution to function as CD19⁺ aAPC. The CD19RCD28 transgene is a second-generation CAR that activates T cells via chimeric CD28 and CD3- ζ . The aAPC (clone #4) were manufactured as a Master Cell Bank by Production Assistance for Cellular Therapies (PACT) under the auspices of the National Heart, Lung, and Blood Institute (NHLBI, Bethesda, MD). Subsequently, a Working Cell Bank of clone #4 was derived at MDACC and used to support numeric expansion of CAR⁺ T cells as cultured in the presence of soluble recombinant human IL-2 and IL-21. The validation studies describing phenotype and function of the CAR⁺ T cells manufactured in compliance with current good manufacturing practice (cGMP) are being published (Singh *et al.*, 2012). In the event there is an overgrowth of CD3^{neg}CD56⁺ lymphocytes early in the culturing process, these cells will be removed using CD56-specific monoclonal antibody and paramagnetic selection. The electroporation and propagation of clinical-grade T cells will occur at the MDACC in compliance with cGMP for Phase I/II trials. The release criteria, undertaken in compliance with Clinical Laboratory Improvement Amendments (CLIA), for the manufactured T cells are (1) sterility (bacteria, fungi, mycoplasma, endotoxin), (2) chain of custody (low-resolution MHC class I typing), (3) phenotype (presence of T cells [CD3 expression], presence of transgene [CAR expression], absence of aAPC [CD32 expression], absence of B cells [CD19 expression]), (4) safety (absence of autonomous cell growth), and (5) viability.

Peripheral blood stem cell mobilization and collection

The methods of peripheral blood stem cell (PBSC) mobilization, collection, storage, and infusion have been described

(Hosing *et al.*, 2006). Patients will receive nonpurged autologous HSC collected after mobilization with filgrastim and chemotherapy. The target progenitor cell dose is 4×10^6 CD34⁺ cells/kg with a minimal acceptable dose of 2×10^6 CD34⁺ cells/kg. Patients failing to reach that target cell number can undergo bone marrow harvest at the discretion of the treating physician. Bone marrow will be obtained by multiple aspirations from the right and left iliac crest under general anesthesia, with a target total nucleated cell dose of 3×10^8 cells/kg. All products are cryopreserved according to standard institutional techniques.

Conditioning regimen for HSCT

We will use our standard-of-care conditioning regimen as a means to lympho-deplete the patients in an effort to improve the persistence of CAR⁺ T cells. The conditioning regimen consists of intravenous carmustine on day -6 at a (300 mg/m^2) infused over 2 hours, followed by etoposide (200 mg/m^2) infused over 3 hours and cytarabine (200 mg/m^2) infused over 1 hour every 12 hours for 8 doses on days -5 through -2, followed by melphalan (140 mg/m^2) infused over 30 minutes on day -1. Autologous PBSC will be infused on day 0, premedicating only with diphenhydramine. Rituximab at 375 mg/m^2 will be infused on days +1 and +8 following PBSC infusion.

T-cell infusion

Clinical observations suggest that infused T cells sustain proliferation in the lymphopenic recipient through homeostatic mechanisms apparently mediated by the removal of regulatory/suppressor cells as well as increased availability of pro-survival cytokines, and in addition, deleterious immune responses that develop against the CAR may be attenuated (Miller *et al.*, 2005; Till *et al.*, 2008; Kochenderfer *et al.*, 2010). T cells will be infused no sooner than 48 hours and no later than 1 week after PBSC infusion. To enhance patient safety, the cryopreserved T-cell product will be split into two portions. 25% of the product will be infused on the first day, with the remaining T-cell dose infused no sooner than 24 hours and no later than 72 hours after completion of the first portion. Before each of the split doses the recipients will be premedicated with acetaminophen and diphenhydramine. After each portion, patients will be monitored over 4 hours for blood pressure, oxygen saturation, and temperature. Systemic corticosteroids and other immunosuppressive agents are contraindicated. If an AE of grade >2 (common terminology criteria [CTC], version 4) occurs involving cardiopulmonary, hepatic (excluding albumin), gastrointestinal, neurological, or renal toxicity probably or definitely attributed to T-cell administration, the patient will immediately begin corticosteroids. If symptoms do not respond to corticosteroids, then additional immunosuppressive agents may be added.

Subcutaneous recombinant human IL-2 after T-cell infusion

After we have established safety and feasibility of the T-cell dosing in the first two cohorts of the study (Table 1), we will assess whether low-dose IL-2 can support the survival and/or numeric expansion of CAR⁺ T cells in the last two cohorts of the trial. IL-2 will be subcutaneously admin-

TABLE 1. RESEARCH PARTICIPANT ASSIGNMENT TO T CELL DOSING COHORTS

Dose cohort	Single T cell dose ^a	IL-2
Dose level X	$>10^7/\text{m}^2$ but $\leq 5 \times 10^7/\text{m}^2$	No
Dose level A	$>5 \times 10^7/\text{m}^2$ but $\leq 5 \times 10^8/\text{m}^2$	No
Dose level B	$>5 \times 10^8/\text{m}^2$ but $\leq 5 \times 10^9/\text{m}^2$	No
Dose level C	$>5 \times 10^7/\text{m}^2$ but $\leq 5 \times 10^8/\text{m}^2$	Yes
Dose level D	$>5 \times 10^8/\text{m}^2$ but $\leq 5 \times 10^9/\text{m}^2$	Yes

^aT cell dosing, starting at Dose level A, is calculated on the basis of preefreeze counts, and infused over a 2-day split.

istered as a single daily injection at $0.3 \times 10^6 \text{ IU/m}^2$ beginning within 2 days of the last split of the T-cell infusion and continuing for up to 14 daily doses. Patients may be premedicated with acetaminophen and diphenhydramine before each injection. The IL-2 dose will be reduced by 50% if the patient develops a new adverse event of grade >2 (CTC, version 4) involving cardiopulmonary, hepatic (excluding albumin), gastrointestinal, neurological, or renal toxicity probably or definitely attributed to IL-2 administration. IL-2 will be stopped in patients with persistent grade >2 (CTC, version 4) toxicity despite IL-2 dose reduction. Patients taking IL-2 with a grade 4 adverse event will stop IL-2. If the adverse event does not improve to grade <3 within 36 hr of stopping IL-2, then corticosteroids will be started. Isolated injection site skin toxicity attributable to IL-2 administration may be an indication to discontinue IL-2, but not to ablate T cells.

Supportive care

Institutional HSCT guidelines for antimicrobial, antifungal, and antiviral prophylaxis will be followed. Patients will not receive filgrastim to enhance neutrophil recovery unless there is concern for serious infection. Packed red blood cells will be administered to maintain hemoglobin levels $\geq 8 \text{ g/dL}$. Platelet transfusions will be administered to keep platelet counts $\geq 10 \times 10^9/\text{liter}$. All blood products are filtered and irradiated. All research participants who are able to have children must practice effective birth control while on study.

Study assessments

Patients will have an initial assessment to include HLA typing, serology for cytomegalovirus (CMV), HIV, human T-lymphotropic virus (HTLV)-1, HCV, and HBV, as well as peripheral blood (PB) collected before manufacture of CAR⁺ T cells and analyzed for protein expression and genetic profiling. Before initiating the conditioning regimen for HSCT, the patients will undergo institutional standard-of-care evaluations to validate adequate organ functions and standard restaging studies, including bone marrow biopsy and positron emission tomography/computed tomography (PET/CT) scans, as clinically indicated. Immediately after the T-cell infusion (last split dose) and then weekly for 2 weeks, and then at 1, 3, 6, and 12 months, the research participants will receive physical and laboratory evaluations, such as PB for protein expression and genetic profiling, including PCR analyses for the presence of infused T cells. Skewing of the T-cell receptor repertoire may indicate that some infused T cells can preferentially survive and thus the PB will be serially analyzed for emergence of oligoclonal or clonal population(s).

of CAR⁺ T cells. In addition, PB will be obtained to measure cytokine levels (including interferon [IFN]- γ): (1) before T-cell infusion, (2) up to two times within 24 hours of the end of the last T cell split infusion, and (3) up to three times between 1 and 7 days after the end of T cell split infusion. Immunoglobulin (IgG) levels will be checked every 2 weeks during the first month after T-cell infusion, and then monthly for 1 year. Intravenous immunoglobulin (IVIG) will be infused if IgG is less than 700 mg/dL. Routine imaging studies and bone marrow aspirate/biopsies will be obtained for disease at 1, 3, 6, and 12 months after HSCT. If tissue (e.g., bone marrow and/or lymph nodes) is available, then testing will evaluate for the presence (trafficking) of CAR⁺ T cells. The recipients will be actively monitored for 30 days to assess AEs. Expected toxicities with T-cell infusion include, but are not limited to, acute infusion reaction (cytokine release syndrome), tachycardia, chills, fatigue, nausea, headache, neuropathic pain, vomiting, cough, shortness of breath, liver toxicity, hypoalbuminemia, hypocalcemia, fever, pruritus, rash, lymphopenia, neutropenia, leukopenia, tumor lysis syndrome, tumor pain, and/or B-cell dysfunction including low numbers of B cells. Enrolled research participants who receive engineered T cells will be required to participate in long-term follow-up (LTFU) per the guidelines set forth by the FDA's Biologic Response Modifiers Advisory Committee that apply to gene transfer studies. Research participants will be enrolled on the LTFU study at the MDACC (IRB #2006-0676) within a year after completion of the T-cell protocol.

Statistical design

The objective of this study is to identify a single dose of T cells without/with IL-2 that can be tolerated by patients. A maximum of 30 patients at four T-cell dose combinations will be enrolled as described in Table 1. We will enroll the first research participant at Dose Level A and after six T cell infusions have been completed, we will enroll successive cohorts of patients at Dose Levels B, C, and D. We anticipate that not all enrolled patients will receive a T-cell infusion. A patient will be eligible for only one (split over 2 days) T-cell infusion. The maximal tolerated dose (MTD) will be declared as the highest T-cell dose at which we are able to treat at least six patients with the proportion of patients having dose-limiting toxicity (DLT) $<2/6$. DLT is defined as a new adverse event of grade >3 involving cardiopulmonary, gastrointestinal, hepatic (excluding albumin), neurological, or renal toxicity (CTC, version 4) that is probably or definitely related to the infused T-cell product. We will use descriptive statistics to summarize the demographic and clinical characteristics of the patients at each Dose Level. We will also report the number of patients with DLT at each dose level. We will estimate the proportion of times that we are unable to prepare the T-cell product with a 95% confidence interval. With 30 patients we will be able to estimate this proportion with a 95% confidence boundary of, at most, 18%. Patients will be removed from the study for withdrawal of the informed consent/authorization, an increasing or unexpected pattern of toxicity deemed unacceptable by the principal investigator, death, noncompliance with study procedures, inability to receive a T-cell infusion, or study completion at 1 year. We will stop the study if any patient develops a T-cell neoplasia directly attributable to the gene

transfer. All adverse events will be appropriately reported to the MDACC, FDA, regulatory agencies, and facilities involved in manufacturing of T cells.

Discussion

This first-in-human Phase I study will assess the safety and feasibility of using the (1) second-generation CD19CD28 CAR, (2) SB system to express CAR in T cells, and (3) aAPC to propagate CAR⁺ T cells. Notably, it is also among the first to combine infusion of CAR⁺ T cells and HSCT. The trial will also investigate the persistence of the infused T cells and correlative studies will assess recipient T cell characteristics and immunological environment that affect T cell survival. The outcome of this trial will lay the groundwork for our protocol (IND #14577, NIH-OPA #0910-1003, IRB #2009-0525) infusing donor-derived CAR-modified T cells to augment the GVT effect after allogeneic HSCT, which has otherwise limited T cell-mediated antitumor results for patients with advanced B-lymphoid malignancies, such as acute lymphoblastic leukemia (Fielding *et al.*, 2007).

An advantage of combining the SB system with aAPC is efficient integration of plasmid DNA and associated shortened time in tissue culture to recover clinically sufficient numbers of T cells that stably express CAR (Ivics *et al.*, 1997; Geurts *et al.*, 2003; Hackett *et al.*, 2005, 2010; Singh *et al.*, 2008; Manuri *et al.*, 2009; Izsvák *et al.*, 2010). DNA plasmids used for the human application of SB transposition can be manufactured at approximately one-tenth the cost of recombinant retrovirus used to transduce clinical grade T cells, which facilitates implementation of early-phase gene therapy trials. Furthermore, use of the aAPC platform as an "off-the-shelf" reagent improves the manufacturing process by avoiding the procurement and use of allogeneic peripheral blood mononuclear cells as irradiated "feeders" (Turtle and Riddell, 2010).

Multiple infusions of CAR⁺ T cells under the supervision of many investigators have generally been well tolerated (Ertl *et al.*, 2011). However, patients with advanced malignancies enrolled in Phase I studies are often medically fragile and complications can occur. There was a grade 5 SAE on ClinicalTrials.gov identifier NCT00466531 (NIH-RAC #0507-721) at the Memorial Sloan-Kettering Cancer Center (MSKCC, New York, NY) after infusion of autologous T cells retrovirally transduced to express a second-generation CD19-specific CAR in a patient with advanced CLL, who before adoptive immunotherapy received cyclophosphamide for desired lymphodepletion (Brentjens *et al.*, 2010). Unfortunately, the patient died within days of the T-cell infusion, but the direct cause of death was attributed to acute renal failure and secondary cause of death attributed to sepsis and hypotension. An autopsy demonstrated no direct toxicity from the infused T cells (Brentjens *et al.*, 2010) and the trial has since successfully reopened at MSKCC. There has been a toxic death directly attributed to the CAR⁺ T cells in a patient at the National Cancer Institute (NCI, Bethesda, MD) with colon cancer metastatic to the lungs who received autologous HER2-specific T cells expressing a third-generation CAR capable of signaling through CD3- ζ , CD28, and CD137 (Morgan *et al.*, 2010). Immediately after administration of the CAR⁺ T cells, the patient developed sudden respiratory distress and died. Evidence appears to indicate that

low-level expression of ERBB2 on normal lung cells was targeted by the genetically modified T cells, leading to cytokine storm and respiratory distress (Morgan *et al.*, 2010). Although patient safety is of paramount importance, the death of patients in Phase I trials is not unexpected. The careful investigations and timely reporting by investigators at the MSKCC and NCI have helped advance the field of T-cell gene therapy as (1) serial measurements of cytokine (especially IFN- γ) levels before and (immediately) after T-cell infusions may help predict patients at risk for SAEs; (2) inpatient T-cell dose escalation may reduce the risk of SAEs; and (3) asymmetric splitting of the T-cell dose over 2 days provides an observation period and limits the number of cells that can be synchronously activated, leading to SAEs (Heslop, 2010; Junghans, 2010; Kohn *et al.*, 2011).

In contrast to the aforementioned unexpected SAEs, we do expect that the recipients will have impaired humoral immunity due to expected T cell targeting of CD19 on normal B cells. Indeed, this has been demonstrated in the study by Kochenderfer and colleagues (2010). In research participants at high risk of mortality due to lymphoid malignancies, the clinical sequelae of B-cell lymphopenia may be an acceptable side effect of CD19-directed immunotherapy, especially because prolonged loss of normal CD20⁺ B cells in patients after rituximab therapy does not appear to result in clinically significant complications attributable to depleted numbers of normal B cells. B cell numbers and immunoglobulin levels will be serially monitored and IVIG will be administered to patients with low immunoglobulin levels to minimize the risk of infection in this setting.

We do not expect that the use of the SB system will lead to genotoxicity. However, the risk of insertional mutagenesis is common to all current approaches in gene therapy. Genetic correction of X-linked severe combined immunodeficiency resulted in deleterious integration of recombinant retrovirus integration and activation of adjacent proto-oncogenes in HSCs, resulting in cases of apparent T-cell leukemia attributed to vector activation of the *LMO2* gene (Cavazzana-Calvo *et al.*, 2000; Hacein-Bey-Abina *et al.*, 2003). Pyrosequencing analysis of cells derived from genetically modified HSC in patients suggests that early emergence of clonal populations of cells can be detected, raising the possibility of initiating preemptive corrective measures (Brady *et al.*, 2011). Although activation of the *LMO2* proto-oncogene can lead to autonomous proliferation, some T cells contain retroviral integrants near a cancer-associated gene (including *LMO2*) without the onset of leukemia (Wang *et al.*, 2010). It appears that the pattern of genomic integrations in cells that have been genetically modified can predict genotoxicity. HSC transduced with retroviral vectors have been infused into patients, rhesus macaques, and mice and in all cases a nonrandom pattern of vector insertions has been reported, with overrepresentation of integrations in one specific genomic locus, a complex containing the *MDS1* and *EVII* genes (Cattoglio *et al.*, 2007; Metais and Dunbar, 2008; Sellers *et al.*, 2010). Either the *MDS1-EVII* genomic locus is a "hot spot" for murine leukemia virus (MLV) integration in hematopoietic stem and progenitor cells, or progenitor cells that happen to acquire an insertion in this locus have a unique propensity to engraft, persist, expand, and/or contribute to hematopoiesis after transplantation (Metais and Dunbar, 2008). The overall significance of insertional mutagenesis in

HSC for CD19-specific CAR⁺ T-cell therapy is unclear. We have adapted the SB system as an alternative to viral gene transfer, as the former has less risk of transferring biological (viral) contaminants (Voigt *et al.*, 2008), and the integration profile after SB transposition is more favorable compared with retrovirus and lentivirus (Hackett *et al.*, 2010; Ivics and Izsvák, 2010). T cells appear to be a suitable cellular substrate for first-in-human testing SB as a new approach to gene therapy, as hundreds of independent viral transductions of human T cells have been safely accomplished (Bonini *et al.*, 1997).

Undertaking cell and gene immunotherapy trials requires a team of dedicated individuals skilled in (1) immunology, (2) manufacturing in compliance with cGMP, (3) correlative studies, (4) conductance of clinical trials, and (5) regulatory affairs. Novel, early-phase clinical trials such as described herein are necessary in order to improve the clinical status of patients refractory to currently available regimens.

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Author Disclosure Statement

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