

Abbreviated Title: Anti-CD19 CAR PBL

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PROTOCOL TITLE

An Assessment of the Safety and Feasibility of Administering T-Cells Expressing an Anti-CD19 Chimeric Antigen Receptor to Patients with B-Cell Lymphoma

NIH Principal Investigator:

Steven A. Rosenberg, M.D., Ph.D.
Chief of Surgery, Surgery Branch, CCR, NCI
Building 10, CRC, Room 3-3940
9000 Rockville Pike, Bethesda, MD 20892
Phone: 240-760-6218; Email: Steven.Rosenberg@nih.gov

Investigational Agent:

Drug Name:	PG13-CD19-H3 (anti-CD19 CAR) retroviral vector-transduced autologous PBL
IND Number:	13871
Sponsor:	Center for Cancer Research
Manufacturer:	Surgery Branch Cell Production Facility

Commercial Agents: Cyclophosphamide and Fludarabine

PRÉCIS

Background:

- We have constructed a retroviral vector that encodes an anti-CD19 chimeric antigen receptor (CAR) that recognizes the CD19 antigen. This chimeric receptor also contains the signaling domains of CD28 and CD3-zeta. The retroviral vector can be used to mediate genetic transfer of this CAR to T-cells with high efficiency (> 50%) without the need to perform any selection.
- In co-cultures with CD19-expressing target cells, anti-CD19-CAR-transduced T-cells secreted significant amounts of IFN- γ and IL-2.
- We have developed a process for cryopreserving the cell product which may lead to the ability for this product to be manufactured at a central location and shipped to other institutions for treatment of a broader patient population.

Objective:

- Primary objective:
 - With the approval of Amendment S, to determine the safety and feasibility of the administration of cryopreserved anti-CD19-CAR engineered peripheral blood lymphocytes with a non-myeloablative conditioning regimen in patients with B-cell lymphomas.

Eligibility:

- Patients of 18 years of age or older must:
 - Have a CD19-expressing B-cell lymphoma
 - Be a non-responder to, or recurred after one or more standard chemotherapy-containing regimens for their malignancy
 - Currently require treatment due to progressive malignancy
 - Be deemed to be incurable by standard therapy
- Patients may not have:
 - A history of allogeneic stem cell transplantation
 - CNS disease

Design:

- PBMC obtained by leukapheresis (approximately 5.0×10^9 cells) will be cultured in the presence of anti-CD3 (OKT3) and aldesleukin in order to stimulate T-cell proliferation.
- Transduction is initiated by exposure of approximately 1.0×10^8 to 5.0×10^8 cells to retroviral vector supernatant containing the anti-CD19 CAR.
- With the approval of Amendment S, patients will receive fludarabine and cyclophosphamide chemotherapy (NMA) for lymphodepletion, followed by cryopreserved anti-CD19-CAR-transduced T-cells.
- Patients will be followed until disease progression.
- Patients who have responded to treatment and then progress may receive one retreatment.

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Prior to Amendment E

1.1.1.1 Primary Objectives

- Phases I and II:
 - Determine the safety of the administration of anti-CD19-CAR engineered peripheral blood lymphocytes and aldesleukin either with or without a non-myeloablative conditioning regimen in patients with B-cell malignancies.
- Phase II only:
 - Determine if lymphodepletion with fludarabine plus cyclophosphamide given prior to infusion of anti-CD19-CAR-transduced T-cells and aldesleukin can enhance persistence of the anti-CD19-CAR-transduced T-cells.

1.1.1.2 Secondary Objective

- Phases I and II:
 - Determine if the administration of anti-CD19-CAR engineered peripheral blood lymphocytes and aldesleukin either with or without the non-myeloablative conditioning regimen causes regression of B-cell malignancies.

1.1.2 With Approval of Amendments E and F

1.1.2.1 Primary Objective

- Determine the safety and feasibility of the administration of anti-CD19-CAR engineered peripheral blood lymphocytes and aldesleukin with a non-myeloablative conditioning regimen in patients with B-cell malignancies.

1.1.2.2 Secondary Objectives

- Determine the *in vivo* survival of the anti-CD19-CAR-transduced T-cells.
- Determine if the treatment regimen cause regression of B-cell malignancies.

1.1.3 With Approval of Amendment H (this study will no longer include aldesleukin)

1.1.3.1 Primary Objective

- Determine the safety and feasibility of the administration of anti-CD19-CAR engineered peripheral blood lymphocytes with a non-myeloablative conditioning regimen in patients with B-cell malignancies.

1.1.3.2 Secondary Objectives

- Determine the *in vivo* survival of the anti-CD19-CAR-transduced T-cells.
- Determine if the treatment regimen cause regression of B-cell malignancies.

1.1.4 With Approval of Amendment S (this study will include cryopreserved anti-CD19-CAR PBL)

1.1.4.1 Primary Objective

- Determine the safety and feasibility of the administration of cryopreserved anti-CD19-CAR engineered peripheral blood lymphocytes with a non-myeloablative conditioning regimen in patients with B-cell malignancies.

1.1.4.2 Secondary Objectives

- Determine the *in vivo* survival of the cryopreserved anti-CD19-CAR-transduced T-cells.
- Determine if the treatment regimen can cause regression of B-cell malignancies.

1.2 BACKGROUND AND RATIONALE

Adoptive transfer of tumor infiltrating lymphocytes (TIL) is an effective therapy for advanced melanoma⁽¹⁾. Adoptive transfer of T-cells that were transduced with the genes of tumor-antigen-specific T-cell receptors caused regressions of melanoma in some patients⁽²⁾. Another approach to adoptive T-cell therapy is to engineer T-cells to express chimeric antigen receptors (CARs). CARs combine a single chain Fv (scFv) component that is derived from a monoclonal antibody with T-cell activation moieties^(3,4). We have constructed and extensively tested an anti-CD19 CAR. T-cells that are transduced with gamma-retroviruses encoding the sequence of this CAR can specifically recognize and kill CD19-expressing target cells including primary chronic lymphocytic leukemia cells. These anti-CD19-CAR-transduced T-cells also produce the cytokines interferon- γ (INF γ) and IL-2 specifically in response to CD19. We propose to conduct a clinical trial in which gamma-retroviruses encoding the anti-CD19 CAR will be used to transduce T-cells from patients with advanced B-cell malignancies. Initially, the protocol will proceed in a phase I dose escalation design, with three cohorts of n=3 to determine the maximum tolerated dose. In phase I, patients will receive lymphodepleting chemotherapy consisting of cyclophosphamide and fludarabine, anti-CD19-CAR transduced T-cells, and high-dose aldesleukin. Once the MTD has been determined, the study then would proceed to the phase II portion. Patients will be randomized to two treatment arms. Patients assigned to treatment Arm 1 will receive fludarabine and cyclophosphamide chemotherapy in order to induce lymphocyte depletion prior to infusion of the anti-CD19-CAR-transduced T-cells, and high-dose aldesleukin. Patients assigned to treatment Arm 2 will receive anti-CD19-CAR transduced T-cells, and high-dose aldesleukin without chemotherapy. This trial design will allow us to rigorously assess the impact of prior lymphodepletion on the persistence of adoptively transferred T-cells in patients.

With the approval of Amendment S, the protocol will proceed in a phase I dose escalation design, with two cohorts of n=3, including a de-escalation cohort to determine the maximum tolerated dose. Patients will receive a lymphodepleting chemotherapy regimen consisting of cyclophosphamide and fludarabine followed by cryopreserved anti-CD19-CAR transduced T-cells.

With the approval of Amendment V, the protocol will proceed in a phase I dose escalation design, with one cohort of n=6 to determine the maximum tolerated dose. Patients will receive a lymphodepleting chemotherapy regimen consisting of cyclophosphamide and fludarabine followed by cryopreserved anti-CD19-CAR transduced T-cells. In this cohort, the dose of cyclophosphamide will be moderately increased to 500 mg/m².

Lymphodepletion has been proven in multiple murine models to dramatically increase the anti-malignancy activity of adoptively-transferred T-cells⁽⁵⁻⁷⁾. In addition, elimination of other host cells such as myeloid suppressor cells has also been shown to enhance anti-tumor immunity in animal models⁽⁸⁾. Human studies strongly suggest that chemotherapy administered before adoptive T-cell transfer can enhance the activity of adoptively-transferred T-cells⁽¹⁾.

1.2.1 Prior Surgery Branch Trials of Cell Transfer Therapy Using Heterogeneous TIL Plus High-Dose IL-2 Following Non-myeloablative but Lymphodepleting Chemotherapy

The NCI Surgery Branch has extensive experience at treating malignancy by adoptive transfer of autologous T-cells (**Table 1**). The best example of the ability of autologous T-cells to mediate regressions of human malignancy is the adoptive transfer of tumor infiltrating lymphocytes (TIL) for treatment of melanoma⁽¹⁾. Prior to adoptive transfer of TIL, patients are lymphodepleted with the chemotherapy agents fludarabine and cyclophosphamide. Patients are lymphodepleted because in most murine models demonstrating the therapeutic effectiveness of adoptively transferred lymphocytes at mediating tumor regression, immunosuppression of the host prior to the adoptive transfer of lymphocytes was required⁽⁹⁾. Interestingly, a clinical trial to formally demonstrate the impact of immunosuppression on adoptively transferred T-cells in humans has not been reported. TIL were cultured from excised melanoma tumors. TIL were then expanded using the rapid expansion protocol (REP) in which TIL were cultured with OKT3, irradiated allogeneic feeder cells, and IL-2. These REPed TIL retained highly specific in vitro anti-tumor activity, often recognized several antigenic epitopes, and contained both CD8+ and CD4+ lymphocytes⁽¹⁾. TIL were adoptively transferred by intravenous infusion. Patients subsequently received high-dose IL-2 and some received peptide immunization when the TIL reactivity was against known MART-1 and gp100 peptides. This regimen using REPed TIL in protocol 99-C-0158 resulted in objective cancer regressions in 51% of patients (18 of 35) with metastatic melanoma⁽¹⁰⁾. Some patients achieved a clonal repopulation of anti-tumor lymphocytes that exceeded 80% of their total circulating CD8+ lymphocytes that persisted for months after cell infusion in some cases⁽¹⁾. A summary of the toxicities that occurred in the 35 patients treated with immunosuppression by fludarabine and cyclophosphamide, TIL infusion, and high-dose IL-2 is shown in **Table 2**. The combination of cyclophosphamide and fludarabine was myelosuppressive. Neutrophils nadired on day 10 after chemotherapy at $6/\text{mm}^3$ and recovered to above $500/\text{mm}^3$ on day 14 with support from filgrastim (G-CSF). Lymphocytes nadired at $6/\text{mm}^3$ and recovered to above $500/\text{mm}^3$. Platelets nadired at $5.5/\text{mm}^3$ on day 8 and recovered to $> 20,000/\text{mm}^3$ on day 28. Patients were usually discharged between 2-3 weeks after the initiation of the chemotherapy. CD4 counts remained persistently low (below 200), which is a known side effect of immunosuppression from fludarabine.

1.2.2 Surgery Branch Trials of Cell Transfer Therapy Using Transduction of TCR Genes that Encode TCR that Recognize Tumor-Associated Antigens into Non-Reactive PBL

Transfer of antigen specific TCR genes to PBL has recently been described as a potential method of generating large numbers of reactive T-cells for anti-cancer therapy^(11, 12). Retroviral vector mediated gene transfer can be used to engineer human T-cells with high efficiency. In published work, the Surgery Branch was among the first to demonstrate that retroviral vector-mediated transfer of TCR genes could endow human PBL with anti-tumor reactivity⁽¹³⁾. In this study, PBL were engineered with a retroviral vector expressing a TCR gene derived from a MART-1

reactive CTL. These engineered cells reacted with MART-1 expressing cells in an HLA-A0201 restricted manner.

Based on this technology, the Surgery Branch currently has conducted five TCR gene transfer trials that have enrolled over 100 patients. In these protocols, patients with metastatic cancer who are HLA-A2 positive received a non-myeloablative but lymphodepleting preparative regimen consisting of cyclophosphamide and fludarabine, and then were treated with autologous peripheral blood lymphocytes or TIL that were genetically engineered to be reactive with melanoma tumor antigens gp100 or MART-1, or with p53. Following adoptive cell transfer, all patients received high-dose IL-2, and some patients received peptide vaccination. In four studies (04-C-0181, 04-C-0251, 07-C-0174, and 07-C-0175) patients with metastatic melanoma are being evaluated, and in one study (07-C-0003), patients with metastatic cancer whose tumors overexpress p53 are being evaluated. **Table 1** provides a summary of these studies.

To test the *in vivo* efficacy of the MART-1 TCR engineered T-cells in 04-C-0251, 17 HLA-A*0201 patients with progressive metastatic melanoma were selected for treatment⁽²⁾. All patients were refractory to prior therapy with IL-2. T-cell cultures from all 17 patients were biologically reactive, with specific secretion of interferon- γ following co-culture with MART-1 peptide pulsed T2 cells and melanoma cell lines expressing the MART-1 antigen. Gene transfer efficiencies in these lymphocytes ranged from 17% to 67% (mean value 42%). Patients received adoptive cell transfer (ACT) with MART-1 TCR transduced autologous PBL after lymphodepletion with fludarabine and cyclophosphamide. An initial cohort of three patients was treated with cells following an extended culture period of 19 days, at which point they had cell doubling times ranging from 8.7 to 11.9 days. In these patients, less than 10% of the transduced cells persisted across the time points tested during the first 30 days post-infusion and 2% or less persisted beyond 50 days. These first three patients showed no delay in the progression of disease. In an effort to administer gene-modified lymphocytes that were in their active growth phase, the culture conditions were modified to limit the *ex vivo* culture period to between 6 and 9 days after stimulation of cells with the anti-CD3 antibody OKT3. In a third cohort, larger numbers of actively dividing cells for ACT were generated by performing a rapid expansion protocol 8-9 days after the initial OKT3 stimulation. In contrast to the lack of cell persistence seen in Cohort 1, patients in cohorts 2 and 3 all exhibited persistence of the transduced cells at greater than 9% at one and four weeks post-treatment (range 9%-56%). All 13 patients examined had increased MART-1 tetramer-binding cells post-treatment and 11 of 14 had increased number of elispot positive cells⁽²⁾.

Most importantly, four patients demonstrated a sustained objective regression of their metastatic melanoma assessed by standard RECIST criteria, two of the responding patients were reported in Morgan et al.⁽²⁾ as patients 4 and 14 (**Figure 1**). In responding patients 4 and 14, gene marked cells in the circulation (assumed to be 1% of total body lymphocytes) expanded 1400 fold and 30 fold respectively compared to the infusion cell number. At one year post-infusion, both responding patients had sustained high levels (between 20%-70%) of circulating gene-transduced cells (**Figure 1E**). The transduced TCR included V β 12, and these two patients displayed V β 12 cells detectable by antibody staining between 12%-16% when followed out to >300 days post-treatment (**Figure 1F**). The responding patients 4 and 14 were also 2 of 4 patients who had greater than 1% circulating tetramer positive cells at greater than 15 days after cell infusion, and these patients demonstrated anti-TAA reactivity in *ex vivo* co-culture assays. On this clinical trial of TCR gene therapy, there were no toxicities in any patient attributed to the gene-marked cells.

Our two newest TCR studies investigate more potent TCRs which target gp100 and MART-1 and expand our studies to TCR-transduced TIL, in addition to PBL. The anti-gp100(154) TCR transduced PBL/TIL study (07-C-0174) has accrued 19 patients to date, all to the TCR transduced PBL arm. At this time, 17 patients have been evaluated for response with 3 patients experiencing PRs and 14 patients having progressive disease. Nineteen patients have been evaluated for toxicities, and most grade 3 and 4 toxicities are known toxicities of the research. Toxicities attributable to the cells include grade 1 and 2 rash, uveitis, dizziness and hearing loss. Two grade 3 toxicities (headache and vasovagal syncope) and one grade 4 event of thrombosis were unexpected events but unrelated to the research. No grade 5 events have been observed on this study. The anti-MART-1 F5 TCR transduced PBL/TIL study (07-C-0175) has accrued 22 patients to date (21 patients to the PBL arm and 1 patient to the TIL arm). At this time 20 patients have been evaluated for response with six patients experiencing a partial response. Twenty-two patients have been evaluated for toxicities, and most grade 3 and 4 toxicities are known toxicities of the research. Toxicities attributable to the cells included grade 1 and 2 rash, uveitis, dizziness, tinnitus, and hearing loss. Five grade 3 events of pain, two grade 3 neurologic toxicities (1 encephalopathy and 1 cerebrovascular ischemia) and 2 grade 4 toxicities (2 pulmonary embolisms) were unexpected events but unrelated to the research. Four grade 3 events of headache were also observed and two of these events were expected and possibly related to the research. No grade 5 events have been observed on this study.

1.2.3 Chimeric Antigen Receptors (CARs)

Targeting T-cells to tumors by transferring the genes for $\alpha\beta$ T-cell receptors into the T-cells of cancer patients for use in adoptive transfer therapies is a promising approach to cancer immunotherapy. Another approach to adoptive T-cell therapy is to engineer T-cells to express chimeric antigen receptors (CARs). CARs are made up of a single chain variable fragment (scFv) coupled to signaling molecules that can activate the T-cells expressing the CAR^(3, 4, 14). The earliest work conducted with CARs was performed in the late 1980s^(15, 16). CARs have been designed to target many different tumor antigens and many important questions have been addressed using CARs in mouse models. Murine models have shown that syngeneic T-cells transduced with retroviruses encoding CARs protected mice from tumor challenges *in vivo*^(17, 18). Murine models indicated that including the signaling domain of CD28 in CARs enhanced tumor protection and persistence of CAR-transduced T-cells^(18, 19). Increasing the number of CAR-transduced T-cells administered to mice consistently enhanced tumor protection as well^(18, 20, 21). One murine study reported that administration of high-dose IL-2 enhanced tumor protection by CAR-transduced T-cells⁽²¹⁾.

Our group has completed a phase I clinical trial in which ovarian carcinoma was treated with T-cells that were transduced with a CAR that was specific for the ovarian carcinoma-associated-antigen α -folate receptor (FR)⁽²²⁾. This trial consisted of two cohorts. In Cohort 1, transduced T-cells that had been activated with OKT3 and transduced with an anti-FR CAR were adoptively transferred to patients. The patients then received a course of high-dose IL-2. In Cohort 2, PBMC of patients were stimulated with allogeneic PBMC and then transduced with the anti-FR CAR. The cells were adoptively transferred to the patients. Next, the patients received injections of allogeneic PBMC from the same donor used for the *in vitro* stimulations as a vaccine. High levels of T-cell transduction were not achieved in this study. No objective clinical responses were attained and the persistence of the transduced T-cells was generally limited to a few days⁽²²⁾. Our currently proposed trial differs from this trial in many ways. First, we can achieve

much higher levels of T-cell transduction due to advances in gene transfer technology. Second, the CAR to be used in our proposed trial incorporates a CD28 signaling domain and the CD3-zeta signaling domain. In contrast the anti-FR CAR used in the earlier work did not contain a CD28 signaling domain and used an Fcγ signaling domain instead of the CD3-zeta moiety in our current receptor. The T-cell culture conditions used in the anti-FR CAR trial are significantly different than the T-cell culture conditions that we will use in our proposed trial of an anti-CD19 CAR. Finally, some of the patients in our proposed trial will be immunosuppressed prior to adoptive transfer of anti-CD19-CAR-transduced T-cells. In the earlier trial no immunosuppression was used.

Another group has reported preliminary results of a clinical trial of a CAR that is specific for carboxy-anhydrase-IX (CAIX)⁽²³⁾. CAIX is expressed by renal cell carcinoma and on bile duct epithelium. The three patients treated on this trial all developed liver toxicity that was attributed to the anti-CAIX-CAR-transduced T-cells⁽²³⁾.

1.2.4 B-Cell Malignancies

Annually in the United States, approximately twenty-two thousand people die of B-cell malignancies⁽²⁴⁾. B-cell malignancies have quite heterogeneous clinical manifestations and prognoses. Chronic lymphocytic leukemia (CLL) is a common disease that is incurable by chemotherapy⁽²⁵⁾. Patients with CLL that is purine-refractory/resistant (defined as no response to a purine-based chemotherapy regimen or progression in 6 months or less after receiving such a regimen) have a median overall survival of 9-13 months⁽²⁶⁾. Patients that have progressive disease greater than 6 months after receiving a purine-based regimen generally have a median overall survival of 3.5 years or less⁽²⁷⁾. Another B-cell malignancy for which new therapies are urgently needed is mantle cell lymphoma. This disease is almost always incurable by chemotherapy and has an aggressive course that is characterized by short responses to chemotherapy⁽²⁸⁾. Follicular lymphoma is a common lymphoma with an extremely variable course⁽²⁹⁾. Patients with follicular lymphoma have a median survival of about 4.5 years after first relapse^(30, 31). Large cell lymphoma is often curable by chemotherapy, and autologous stem cell transplants are sometimes able to cure patients with relapsed large cell lymphoma⁽³²⁾. However, patients with multiple relapsed large cell lymphoma have very poor prognoses and some of them are candidates for experimental therapies⁽³³⁾. Allogeneic stem cell transplantation is a potentially curative treatment option for patients with advanced B-cell malignancies, but allogeneic stem cell transplantation generally has a treatment related mortality rate of 10-30%^(34, 35). Improved therapies for B-cell malignancies are clearly needed.

Our proposed trial makes use of fludarabine and cyclophosphamide to lymphodeplete the patients assigned to one of the two treatment arms. Because of the activity of fludarabine and cyclophosphamide against B-cell malignancies^(26, 29, 32), we will not be able to determine whether tumor regressions that occur on this arm of the trial are due to the chemotherapy or to the adoptively transferred T-cells or a combination of both chemotherapy and T-cells. Our group has previously used IL-2 alone in the same dose and schedule proposed for this trial as a treatment for lymphoma⁽³⁶⁾. No objective responses were observed in the eleven lymphoma patients treated with IL-2 alone⁽³⁶⁾. Therefore, any responses that take place in the arm of our proposed trial in which patients do not receive chemotherapy can reasonably be attributed to the infused anti-CD19-CAR-transduced T-cells.

1.2.5 CD19

We have constructed a CAR that recognizes CD19. We chose to target CD19 because it is expressed on most malignant B-cells^(37, 38), but the only normal cells that express CD19 are B-cells and perhaps follicular dendritic cells^(38, 39). Importantly, CD19 is not expressed on pluripotent hematopoietic stem cells⁽⁴⁰⁾. While destruction of normal B-cells is a drawback to targeting CD19, several factors indicate that destruction of normal B-cells is tolerable. When patients receive the anti-CD20 monoclonal antibody rituximab, the number of normal B-cells is severely depressed for several months⁽⁴¹⁾, yet patients that receive chemotherapy plus rituximab do not have an increased rate of infections when compared to patients that receive chemotherapy alone⁽⁴²⁾. Finally, patients can be treated with intravenous infusions of IgG if necessary to increase IgG levels⁽⁴³⁾.

1.2.6 Development of an Anti-CD19 CAR for Clinical Adoptive T-Cell Transfer

The anti-CD19 CAR used in our work consists of three main components: the variable regions of the anti-CD19 monoclonal antibody FMC63⁽⁴⁴⁾, part of the CD28 costimulatory molecule, and the signaling domain of the CD3 zeta chain⁽⁴⁵⁾. This receptor is referred to as FMC63-28. The DNA encoding this receptor was cloned into the MSGV1 retroviral vector backbone⁽²⁾ to form the plasmid shown in **Figure 2A**. This plasmid is referred to as MSGV1-FMC63-28. For the remainder of this document the FMC63-28 receptor will be referred to as the anti-CD19 CAR.

We carried out extensive in vitro experiments to assess the suitability of the FMC63-28 anti-CD19 CAR for use in a clinical trial. In order to transduce T-cells with the anti-CD19 CAR, we first suspended normal peripheral blood mononuclear cells (PBMC) in IL-2-containing media and stimulated them with the anti-CD3 monoclonal antibody OKT3. Two days after the OKT3 stimulation was initiated, we spin-loaded gamma-retroviruses encoding the anti-CD19 receptor onto a retronectin-coated plate. The OKT3-activated PBMC were placed on the plate and cultured overnight. The next day, we transferred the OKT3-activated PBMC from the original virus-coated plate to a second plate that had been loaded with retroviruses encoding the anti-CD19 CAR in a manner identical to the first transduction, and the cells were cultured overnight again. Preliminary experiments using transiently produced retroviral supernatant for transductions showed that our FMC63-28 receptor functioned well in vitro; therefore, a stable producer cell clone that produces gibbon ape leukemia virus (GALV) pseudotyped retrovirus encoding the FMC63-28 anti-CD19 CAR was generated. We used supernatant from this clone for the rest of the experiments described in this document. Following transduction, the PBMC were almost 100% CD3⁺ T-cells. Because the anti-CD19 CAR contains variable regions that were derived from a murine antibody, a goat-anti-mouse-Fab antibody can stain cells that express the anti-CD19 CAR. An example of staining of anti-CD19-transduced T-cells with the anti-Fab antibody and CD3 is shown in **Figure 2B**. In six separate transduction experiments, a mean of 57% of CD3⁺ T-cells expressed the anti-CD19 CAR. These transduced cells produced interferon- γ (IFN γ) specifically in response to CD19-expressing target cell lines (**Figure 3**).

Next, we obtained PBMC from a patient with CLL that had previously been treated with fludarabine and rituximab. We stimulated these PBMC with OKT3 in IL-2-containing media and transduced them with retroviruses encoding the anti-CD19 CAR. We found that the T-cells from this patient proliferated well in response to OKT3 and that 54% of the CD3⁺ cells expressed the anti-CD19 CAR. In addition, these transduced cells produced IFN γ in response to primary

allogeneic CLL cells, but not CD19-negative target cells and they killed primary allogeneic CLL cells in an in vitro cytotoxicity assay⁽⁴⁶⁾ (**Figure 4**).

In order to obtain the large number of T-cells necessary for clinical adoptive T-cell transfer, T-cells that have been stimulated with OKT3 and transduced are subjected to a second stimulation with OKT3 in the presence of allogeneic feeder cells in IL-2-containing media. This process is called a rapid expansion protocol (REP). The data presented in **Figure 5A** demonstrate that after being subjected to a REP, anti-CD19-CAR-transduced T-cells produce IFN γ in response to CD19-expressing target cells but not in response to CD19-negative cells. The cells can also kill primary CLL cells (**Figure 5B**). CD19-CAR-transduced T-cells produce IFN γ and IL-2 in response to target cells that were transduced with CD19, but not target cells that were transduced with the control antigen nerve growth factor receptor (NGFR) (**Figure 6**).

Like most T-cells, anti-CD19-CAR-transduced T-cells are dependent on exogenous IL-2 for survival in vitro (**Figure 7**). We have demonstrated IL-2-dependent in vitro survival of anti-CD19-CAR-transduced T-cells in many experiments. These results provide strong evidence that the transduced T-cells do not become immortalized.

1.2.7 Other Anti-CD19 CAR Clinical Trials

Other groups have constructed anti-CD19 CARs^(20, 47). Jensen and coworkers have completed a clinical trial of adoptive transfer of T-cells that were transfected with a plasmid encoding an anti-CD19 chimeric receptor⁽⁴⁸⁾. The T-cells expressing the anti-CD19 receptor did not persist *in vivo* and did not cause objective remissions of lymphoma. Our proposed clinical trial differs from the completed trial of Jensen and coworkers in many ways. The most important difference is that our proposed trial will use a retroviral vector rather than plasmid transfection to transfer an anti-CD19 receptor to T-cells. Another potentially important difference is that the CAR we propose to utilize in our clinical trial contains the signaling component of CD28, but the receptor construct used by Jensen and coworkers did not include a CD28 moiety.

A second group is currently conducting a clinical trial of adoptive transfer of T-cells that have been transduced with retroviruses encoding an anti-CD19 receptor (NCI Clinical Trials PDQ, trial MSKCC trial #06-138, Principal Investigator: R. Brentjens). This trial is enrolling only CLL patients. In this trial, CAR-transduced T-cells are transferred to patients that have received either cyclophosphamide chemotherapy or no chemotherapy prior to cell transfer. Our proposed trial differs from the trial of Brentjens and coworkers in several ways. First, we plan to administer high-dose IL-2 after cell transfer. Second, we propose to randomize between administering fludarabine and cyclophosphamide chemotherapy prior to adoptive T-cell transfer or administering no chemotherapy prior to T-cell transfer. Finally, we will treat all CD19-expressing malignancies while Brentjens and coworkers are only treating CLL.

Malcolm Brenner, Helen Heslop, and coworkers are conducting a clinical trial in which anti-CD19-CAR-transduced T-cells are administered to patients with indolent B-cell malignancies (NCI Clinical Trials PDQ, trial #BCM-H-19384). In this trial, each patient is receiving a mix of T-cells in which some T-cells are transduced with a CAR that incorporates a CD28 moiety and other T-cells are transduced with a CAR lacking a CD28 moiety. The aim of the trial is to evaluate the importance of the CD28 moiety on T-cell persistence and function. Our proposed trial differs from this trial significantly. First, this trial uses no immunosuppression prior to cell infusion while our trial proposes to randomize between immunosuppression with fludarabine

plus cyclophosphamide versus no immunosuppression. Second Brenner, Heslop, and coworkers are not administering IL-2 as part of their trial.

With the approval of Amendment S, patients will receive a lymphodepleting chemotherapy regimen consisting of cyclophosphamide and fludarabine followed by cryopreserved anti-CD19-CAR transduced T-cells.

1.2.8 Rationale for Measuring Persistence of Adoptively Transferred T-Cells With or Without Lymphodepleting Chemotherapy Prior to Adoptive Transfer

We hypothesize that lymphodepletion with fludarabine and cyclophosphamide will enhance persistence of the adoptively transferred anti-CD19 T-cells. Murine studies demonstrate enhanced anti-tumor efficacy of transferred T-cells in lymphodepleted hosts compared to lymphoreplete hosts⁽⁴⁹⁾. However, in previous nonrandomized Surgery Branch trials, melanoma patients were treated with tumor infiltrating lymphocytes (TIL) plus IL-2 preceded by either one dose of 25 mg/kg of cyclophosphamide or no chemotherapy⁽⁵⁰⁾. There was no difference in the objective tumor response rates when patients that received chemotherapy prior to adoptive T-cell transfer were compared to patients that did not receive chemotherapy⁽⁵⁰⁾. Our proposed trial will be the first randomized trial to evaluate the impact of immunosuppression on the persistence of adoptively transferred T-cells in humans. Persistence of adoptively transferred T-cells has been shown to be associated with anti-tumor efficacy⁽⁵⁾. An additional benefit of not treating some patients on this trial with chemotherapy is that the anti-malignancy effect of anti-CD19-CAR-transduced T-cells plus IL-2 can be evaluated without the confounding effect of chemotherapy.

In the past few years a large amount of evidence has been accrued demonstrating that lymphodepletion prior to cell infusion is critical for the effectiveness of adoptive T-cell transfer^(5, 49-51). Much of this evidence has been elicited since this protocol was originally submitted. Therefore, starting with Amendment E, patients will no longer be treated with CD19-CAR engineered cells in the absence of lymphodepletion.

1.2.9 Safety Considerations

Several safety concerns regarding the infusion of large numbers of retrovirally modified tumor reactive T-cells have been addressed in our previous clinical studies. The non-myeloablative chemotherapy and the administration of high-dose IL-2 have expected toxicities discussed earlier. The immuno-myeloablative chemotherapy used in this protocol has been administered to over 100 patients and all have reconstituted their hematopoietic systems.

In other protocols we have administered over 3.0×10^{11} TIL with widely heterogeneous reactivity including CD4, CD8, and NK cells without difficulty. As discussed above, the expansion of tumor reactive cells is a desirable outcome following the infusion of antigen reactive T-cells. Some patients receiving gp100 or MART-1 reactive cells have developed vitiligo, uveitis, hearing loss and rash probably due to destruction of normal melanocytes though these toxicities have been manageable. In addition, two patients experienced vestibular dysfunction possibly due to the transduced cells. We do not believe the transfer of these gene modified cells has a significant risk for malignant transformation in this patient population. While the risk of insertional mutagenesis is a known possibility using retroviral vectors, this has only been observed in the setting of infants treated for XSCID using retroviral vector-mediated gene transfer into CD34+ bone marrow cells. In the case of retroviral vector-mediated gene transfer into mature T-cells, there has been no evidence of long-term toxicities associated with these

procedures since the first NCI sponsored gene transfer study in 1989. Although continued follow-up of all gene therapy patients will be required, data suggest that the introduction of retroviral vectors transduced into mature T-cells is a safe procedure. While we believe the risk of insertional mutagenesis is extremely low, the proposed protocol follows all current FDA guidelines regarding testing and follow up of patients receiving gene transduced cells.

As of April 2015, thirty-four patients have been enrolled on this study. This includes seven patients with CLL, four patients with follicular lymphoma, one patient with splenic marginal zone lymphoma, fifteen patients with diffuse large B-cell lymphoma, five patients with primary mediastinal B-cell lymphoma, one patient with non-Hodgkin lymphoma, and one with Mantle cell lymphoma. We have observed impressive clinical response rates of 70% in this study, which includes 12 patients with a partial response (PR) and 12 patients with a complete response (CR) to treatment. However, 11 patients on this study have experienced a dose limiting toxicity (DLT) resulting in the trial being placed twice on clinical hold by the FDA. Subsequently, the dose was reduced three times, IL-2 was eliminated, and the dose of cyclophosphamide was decreased by 50%. The most common toxicities observed were neurotoxicities (somnolence, aphasia, and tremors), hypotension and one incidence of renal failure. Analysis of blood samples on these patients has indicated the elevated levels of cytokines which are the likely cause of these events.

1.2.10 Cryopreservation of the Anti-CD19 CAR Transduced T-Cell Product

As noted above, we have seen substantial clinical activity in patients with B-cell lymphomas receiving these anti-CD19 gene transduced cells. The cells have most often been administered following approximately 10-12 days of growth and were given from freshly harvested cultures. This procedure involves multiple logistic problems especially if we are to develop a procedure that can be more widely disseminated. Difficulty in timing the lymphodepleting chemotherapy with completion of the culture and the need to ship cells to other institutions in subsequent protocols has led us to slightly alter the standard operating procedures for growth of cells. In the current protocol we have developed a protocol for developing cells following just six days of culture. The cells are then cryopreserved and immediately infused following thawing. We have done substantial comparability studies to show that the cells grown for six days and cryopreserved have the same phenotype and functional properties as cells that have grown for longer period of times and administered fresh. Thus, in the next cohort of patients we plan to use cells grown for six days in culture and then cryopreserved and thawed prior to administration.

In an effort to establish a more GMP-compliant cell production process, we developed a closed cell production resulting in a cryopreserved cell product at the end of 6 days. A schematic showing the process flow for the closed process is presented in [Figure 8](#). Briefly, 1.0×10^9 PBMC are stimulated in a bag with soluble OKT3 for 2 days. The stimulated PBMC (1X) are washed on a Sepax using the neat cell wash program and transferred to retronectin-coated bag containing gammaretroviral vector supernatant (1X) resulting in a final cell density of 2.0×10^8 cells in 2X diluted vector supernatant. After an overnight incubation at 37°C, the cells are transferred to an expansion bag containing Optimizer medium and 2.5% TCSR at a density of 0.5×10^6 cells/mL. The cells are expanded for 4 days, washed on the Sepax and then cryopreserved in bags at a concentration of 2.0×10^6 CAR+ cells/kg in 50 mL of 50% Saline-2.5% HSA-50% Cryostor-10.

We conducted 5 engineering runs at scale using cells from lymphoma patients in parallel with our current open clinical cell production process in order to assess the comparability of the closed process in terms of transduction efficiency, cell expansion and total cell number,

biological function as measured by IFN γ release following coculture with antigen-positive targets, and phenotype. The only significant difference detected between the two processes was the level of transduction. Cells transduced in the closed cell production process exhibited a significantly lower transduction efficiency as compared to the open system (**Figure 9**, $43.6 \pm 8.3\%$ and $79.6 \pm 1.5\%$, $n=5$, respectively ($p<0.001$). We were able to achieve comparable total cell numbers (**Figure 10**, $14.5 \pm 4.5 \times 10^8$ and $19.6 \pm 13.6 \times 10^8$ total cells, respectively) as well as cell expansion (**Figure 11**, 8.2 ± 2.1 and 11.4 ± 5.0 fold expansion, respectively) using the 6 day cell production process. In addition, cells manufactured using the 6 day closed process are CD19-specific as evidenced by release of comparable levels of IFN γ when cocultured with CD19+ targets (Nalm6, Toledo) and not CD19- targets (K562-NGFR, CEM), (**Figure 12**). The last measure of comparability between cells generated using the 6 day closed process to those generate in our current open clinical process is based on T-cell phenotype (**Figure 13**). There was no significant difference in T-cell phenotype based on detection of CD45RA and CCR7 by FACS.

Having shown that the 6-day cell production process can yield a cell product that is comparable to our current 10-day open clinical production process, we next sought to determine what effect cryopreservation had on our T-cell product. To assess the effect of cryopreservation on the percentage of CAR+ T-cells, cell viability, expansion, function and phenotype (**Figure 17**), we conducted two additional engineering runs using cells from lymphoma patients. Where possible the 6-day cell product was evaluated pre- and post-thaw for a given parameter. In **Figure 14A**, we demonstrate that there is no significant difference in the percentage of CAR+ T-cells 3 days post-thaw suggesting that our cryopreservation protocol is not inherently detrimental to CAR expression. In addition, the CAR+ cells continued to show CD19-specific antigen recognition as measured by IFN γ release following coculture with CD19+ targets (**Figure 15A** and **Figure 15B**). The viability of the cells at thaw was 90 and 79%, respectively, for the two patients tested (**Figure 14B**). Interestingly, the viability of the cells dipped 24h post-thaw by an average of 14% (**Figure 16A**); however, the cells then quickly recovered by the next day and expanded 36- and 21-fold over a period of 7 days in the absence of an antigen-specific stimulation (**Figure 16B**). There were slight differences in T-cell phenotype following cryopreservation, but it is likely that these differences are not specific given that the FACS staining was done on different days. It is important to note that while the percentages of a particular T-cell subset might change for a given patient, there is no difference in the profile of the subsets for each patient. Thus, the cells appear comparable following cryopreservation and thaw as compared to the pre-cryopreserved cells.

As of April 1, 2014, twenty-six patients have been enrolled on this study; this includes seven patients with CLL, four patients with follicular lymphoma, one patient with splenic marginal zone lymphoma, and fourteen patients with diffuse large B-cell lymphoma. Of the twenty-six patients treated on this study, we have observed impressive clinical response rates of 77%, which include 10 patients with a partial response and 10 patients with a complete response to treatment.

The protocol was initially designed as a standard dose escalation study with the dose escalation based on our prior experience with cell therapy. Due to the dose limiting toxicities, we have modified the protocol accordingly; eliminating aldesleukin, reducing the cell dose, and decreasing the doses of both chemotherapy agents. Some of the patients treated in the first few groups had severe side effects, including difficulty breathing, inability to speak, confusion, tremors, and kidney damage.

In Cohort 1, eight patients were treated with high-dose chemotherapy (cyclophosphamide (60mg/kg x 2 days) and fludarabine (25mg/m² x 5 days)) followed by anti-CD19 CAR T-cells (between 4.0x10⁸ and 4.0x10⁹ cells) and high dose aldesleukin. Of these 8 patients, 7 are evaluable, one of these patients died on study due to complications related to treatment and H1N1 infection. Six of the seven evaluable patients have objective responses; 4 partial responders and 2 complete responders. Due to severe toxicities, this cohort was closed to evaluate the safety and toxicity of lower cell doses and no aldesleukin.

In Cohort 6, two patients were treated with a high-dose chemotherapy followed by anti-CD19 CAR+ cells (0.5x10⁷ transduced cells/Kg) and no IL-2. Both patients have achieved a complete response (CR). This cohort was closed due to grade 3 neurological toxicities, so we added a dose de-escalation cohort to continue accrual.

In Cohort 8, five patients were treated with high-dose chemotherapy followed by anti-CD19 CAR+ cells (2.5x10⁶ transduced cells/Kg) and no IL-2. One patient died due to disease and treatment (not evaluable). Three patients have achieved a complete response (CR) and one patient is a non-responder. This cohort was closed due to grade 4 neurological toxicities observed in two patients, so the dose of cyclophosphamide was reduced to 30mg/kg/day for 2 days and the dose of cells was reduced to 1.0x10⁶ transduced cells/kg.

In Cohort 9, six patients were treated with reduced cyclophosphamide dose (30mg/kg x 2 days) and fludarabine (25mg/m² x 5 days) followed by anti-CD19 CAR+ cells (1.0x10⁶ transduced cells/Kg) and no IL-2. Three patients have achieved a complete response (CR) and two patients have achieved a partial response (PR). This cohort was closed due to grade 4 creatinine and neurological toxicities, therefore decreasing the total dose of cyclophosphamide by more than 50% and fludarabine by about 30%.

In Cohort 10, five patients were treated with reduced doses of cyclophosphamide (300mg/m² x 3 days) and fludarabine (30mg/m² x 3 days) followed by 1.0x10⁶ cells/kg and no aldesleukin. Four patients have confirmed partial responses (PR) and one patient is a non-responder. The majority of toxicities seen in this cohort were attributed to the chemotherapy preparative regimen and none of the toxicities attributed to the fresh cell product were dose limiting toxicities (DLTs).

With the approval of Amendment S, patients will receive Cryopreserved anti-CD19 CAR positive cells/kg followed by cyclophosphamide and fludarabine.

In Cohort 11, four patients were treated with the reduced doses of cyclophosphamide and fludarabine followed by Cryopreserved anti-CD19 CAR+ cells (2.0x10⁶ transduced cells/kg) and no IL-2. Two patients are non-responders. Two patients have achieved partial responses.

In Cohort 12, one patient was treated with the reduced doses of cyclophosphamide and fludarabine followed by Cryopreserved anti-CD19 CAR+ cells (6.0x10⁶ transduced cells /kg) and no IL-2. This patient is a non-responder.

In Cohort 13, one patient was treated with a moderately increased dose of cyclophosphamide and fludarabine followed by Cryopreserved anti-CD19 CAR+ cells (2.0x10⁶ transduced cells /kg) and no IL-2. This patient has achieved a partial response (PR).

In Cohort 11 the 6-day product was cryopreserved in order to evaluate a process which could facilitate commercialization of the product by our CRADA partner. Four patients were treated in Cohort 11 and all but one patient incurred at least grade 2 toxicities. One patient was treated in

Cohort 12; this patient experienced grade 4 confusion requiring transfer to the ICU, one patient was treated in Cohort 13 and he experienced grade 3 confusion and aphasia. In a review of the data it appeared that patients who received fresh products where the cells had been in culture for 9-10 days (9-10 day product) incurred fewer toxicities and at a lesser grade than those patients who had received cryopreserved cells which had been in culture for 6 -8 days. In order to further explore the relationship between days in culture and toxicity, we are increasing the length of time in culture. In Cohort 14 the cells will be in culture for 9 days, which may be increased to 12 days depending upon the characteristics of the product. The product will continue to be cryopreserved and the dose escalation schema will continue as described in Section 3. In addition, in order to establish a baseline which could aid in determining the cause of, or further define, these neurologic toxicities, patients enrolled in Cohort 14 may undergo lumbar puncture for CSF analysis by flow cytometry prior to treatment. Patients who have undergone LP following treatment per protocol have all shown CD19+ cells in their CSF however, it is not known to what degree these cells were present prior to treatment as the patients were all asymptomatic and had nothing remarkable seen on imaging, and thus did not meet the criteria for a lumbar puncture prior to treatment.

Note: The paragraphs highlighted in grey are no longer applicable after the approval of Amendment X.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

- 2.1.1.1 Patients must have a CD19-expressing B-cell lymphoma. Patients with Diffuse large B-cell lymphoma, Primary Mediastinal B-cell lymphoma, and Diffuse large B-cell lymphoma transformed from follicular lymphoma must have measurable disease after at least two prior chemotherapy regimens one of which must have contained doxorubicin and rituximab.
- 2.1.1.2 Confirmation of diagnosis of B-cell malignancy and positivity for CD19 confirmed by the Laboratory of Pathology of the NCI. The choice of whether to use flow Cytometry or immunohistochemistry will be determined by what is the most easily available tissue sample in each patient. Immunohistochemistry will be used for lymph node biopsies, flow Cytometry will be used for peripheral blood, fine needle aspirates and bone marrow samples.
- 2.1.1.3 Patients must have indications for treatment for their B-cell malignancy at the time of enrollment on this trial.
- 2.1.1.4 Greater than or equal to 18 years of age and less than or equal to age 70.
- 2.1.1.5 Willing to sign a durable power of attorney.
- 2.1.1.6 Able to understand and sign the Informed Consent Document.
- 2.1.1.7 Clinical performance status of ECOG 0 or 1.
- 2.1.1.8 Life expectancy of greater than three months.
- 2.1.1.9 Patients of both genders must be willing to practice birth control from the time of enrollment on this study and for four months after treatment.

2.1.1.10 Women of child bearing potential must have a negative pregnancy test because of the potentially dangerous effects of the treatment on the fetus.

2.1.1.11 Serology

- Seronegative for HIV antibody. (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who are HIV seropositive can have decreased immune -competence and thus are less responsive to the experimental treatment and more susceptible to its toxicities.)
- Seronegative for hepatitis B antigen and hepatitis C antibody unless antigen negative. If hepatitis C antibody test is positive, then patients must be tested for the presence of antigen by RT-PCR and be HCV RNA negative.

2.1.1.12 Hematology

- Absolute neutrophil count greater than or equal to $1000/\text{mm}^3$ without the support of filgrastim.
- Platelet count greater than or equal to $50,000/\text{mm}^3$.
- Hemoglobin greater than 8.0 g/dl.
- Lymphocyte count less than or equal to $4,000/\text{mm}^3$.

2.1.1.13 Chemistry

- Serum ALT/AST less or equal to 5 times the upper limit of normal.
- Serum creatinine less than or equal to 1.6 mg/dl.
- Total bilirubin less than or equal to 1.5 mg/dl, except in patients with Gilbert's Syndrome who must have a total bilirubin less than 3.0 mg/dl.

2.1.1.14 More than three weeks must have elapsed since any prior systemic therapy at the time the patient receives the preparative regimen, and patients' toxicities must have recovered to a grade 1 or less (except for toxicities such as alopecia or vitiligo).

2.1.1.15 Normal cardiac ejection fraction and no evidence of pericardial effusion as determined by an echocardiogram.

2.1.2 Exclusion Criteria

2.1.2.1 Patients that require urgent therapy due to tumor mass effects such as bowel obstruction or blood vessel compression.

2.1.2.2 Patients that have active hemolytic anemia.

2.1.2.3 Patients with active brain metastases, or with a history of any CNS metastases or cerebrospinal fluid malignant cells. Note: Patients who are asymptomatic but are found to have malignant cells in the CSF on lumbar puncture prior to treatment will be considered eligible.

2.1.2.4 Women of child-bearing potential who are pregnant or breastfeeding because of the potentially dangerous effects of the treatment on the fetus or infant.

- 2.1.2.5 Active systemic infections, coagulation disorders or other major medical illnesses of the cardiovascular, respiratory or immune system, myocardial infarction, cardiac arrhythmias, obstructive or restrictive pulmonary disease.
- 2.1.2.6 Any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease).
- 2.1.2.7 Concurrent opportunistic infections (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who have decreased immune competence may be less responsive to the experimental treatment and more susceptible to its toxicities).
- 2.1.2.8 Concurrent systemic steroid therapy.
- 2.1.2.9 History of severe immediate hypersensitivity reaction to any of the agents used in this study.
- 2.1.2.10 History of allogeneic stem cell transplantation
- 2.1.2.11 Patients with cardiac atrial or cardiac ventricular lymphoma involvement.

2.2 SCREENING EVALUATION

2.2.1 Within 4 Weeks Prior to Starting the Chemotherapy Regimen

- Complete history and physical examination, including, weight and vital signs, noting in detail the exact size and location of any lesions that exist. (**Note:** patient history may be obtained within 8 weeks.)
- Chest x-ray
- EKG
- Baseline CT of the chest, abdomen and pelvis, PET scan, and brain MRI to evaluate the status of disease. Additional scans and x-rays may be performed if clinically indicated based on patients' signs and symptoms.
- HIV antibody titer and HbsAG determination, and anti HCV, (Note: may be performed within 3 months of the chemotherapy start date).
- Anti CMV antibody titer, HSV serology, and EBV panel (Note: patients who are known to be positive for any of the above do not need to be retested; may be performed within 3 months of chemotherapy start date)
- Patients with a LVEF of less than or equal to 55% will not proceed to treatment, (Note: may be performed within 8 weeks of treatment).
- CD19 staining of malignant cells by immunohistochemistry or flow cytometry (testing is permitted to be conducted at any time prior to this point).
- All patients must have a TBNK for Peripheral blood CD3 count and CD19#.
- Patients with a history of leptomeningeal disease, or signs/symptoms suggestive of leptomeningeal involvement, or with symptoms of central nervous system malignancy such as new onset severe headaches, neck stiffness, or any focal neurologic findings on physical exam will have lumbar puncture for examination of cerebral spinal fluid.

- Patients may undergo lumbar puncture (LP) for flow cytometry of the CSF in order to assess the presence of CD19 positive lymphocytes for potential correlation with neurologic toxicity. Patients who have no neurologic symptoms at the time of LP will be eligible for enrollment regardless of the results of the flow cytometry.

2.2.2 Within 14 Days Prior to Starting the Chemotherapy Regimen

- Chem 20: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO₂ (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid)
- Thyroid panel
- CBC with differential and platelet count
- PT/PTT
- Urinalysis and culture, if indicated

2.2.3 Within 7 Days Prior to Starting the Chemotherapy Regimen

- β -HCG pregnancy test (serum or urine) on all women of child-bearing potential
- ECOG performance status of 0 or 1

2.3 PROTOCOL REGISTRATION

2.3.1 Prior to Registration for this Protocol

Patients will initially be registered on protocol 03-C-0277 (Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols) prior to transduction of PBL cells (either fresh or cryopreserved samples), by the clinical fellow or research nurse. Once cells exceed the potency requirement and are projected to exceed the minimum number specified in the Certificate of Analysis (CoA), patients will sign the consent document for this protocol.

2.3.2 Registration Procedure

Authorized staff must register an eligible candidate with the NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the website (<http://home.ccr.cancer.gov/intra/eligibility/welcome.htm>) must be completed and sent via encrypted email to the NCI Central Registration Office at ncicentralregistration-l@mail.nih.gov. After confirmation of eligibility at the Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol. Verification of registration will be forwarded electronically via email to the research team. A recorder is available during non-working hours.

3 STUDY IMPLEMENTATION

Due to toxicities experienced during the dose escalation phase of this trial, the protocol has been amended several times to decrease the cell dose, eliminate the administration of IL-2, and administer reduced doses of cyclophosphamide and fludarabine. The description of these changes has been moved to [Appendix 2](#) and [Appendix 3](#).

3.1 STUDY DESIGN – BEGINNING WITH AMENDMENT S

Patients with B-cell malignancies will receive a non-myeloablative conditioning regimen consisting of cyclophosphamide and fludarabine chemotherapy followed by cryopreserved anti-CD19 CAR transduced T-cells. Patients will receive one course of treatment. The start date of the course will be the start date of the chemotherapy; the end date will be the day of the first post-treatment evaluation. Patients may undergo a second treatment as described in Section 3.5.

3.1.1 Treatment Phase: Cryopreserved PBL

Opened with Approval of Amendment S

PBMC will be obtained by leukapheresis (approximately 1.0×10^{10} cells). In most patients, whole PBMC will be cultured in the presence of anti-CD3 (OKT3) and aldesleukin in order to stimulate T-cell growth. In some patients the peripheral blood might be contaminated with large numbers of CD19-expressing malignant cells. In patients with peripheral blood lymphocytes counts greater than 4000 cells per microliter, CD19-expressing cells will be depleted using the Miltenyi Biotec CliniMac system with anti-CD19 microbeads and then CD19-depleted PBMC will be stimulated with OKT3. In all patients, 2 days after OKT3 stimulation transduction will be initiated by exposure of approximately 1.0×10^7 to 5.0×10^8 cells to supernatant containing the anti-CD19 CAR retroviral vector. These transduced cells will be expanded and tested for their anti-tumor activity. Successful TCR gene transfer will be determined by FACS analysis for the CAR and specific anti-tumor reactivity will be tested by cytokine release measured against CD19+ cell lines and CD19-negative cell lines. Successful CAR gene transfer for each transduced PBL population will be defined as >30% CAR positive cells and for biological activity, gamma-interferon secretion against CD19 targets must be at least 200 pg/mL. The cells administered vary depending on their growth characteristic. In prior protocols over 3.0×10^{11} T-cells have been safely infused to cancer patients.

Standard Operating Procedures for the cryopreservation and thawing processes will be submitted to BB-IND 13871.

With the approval of Amendment S patients will be lymphodepleted with reduced doses of fludarabine and cyclophosphamide followed by cryopreserved anti- CD19 CAR positive cells/kg ($\pm 20\%$) as noted below.

With the approval of Amendment V, the dose of cyclophosphamide was increased from 300 mg/m²/day x 3 days to 500 mg/m²/day x 3 days. Patients will be lymphodepleted with a modest increase of cyclophosphamide and continue with the current dose of fludarabine, then followed by cryopreserved anti- CD19 CAR positive cells/kg ($\pm 20\%$).

With the approval of Amendment X, the number of days in culture was increased to 9-12. Patients received the current regimen of cyclophosphamide and fludarabine followed by 2.0×10^6 cryopreserved anti- CD19 CAR positive cells/kg ($\pm 20\%$). With the approval of Amendment Z cells may be in culture for 6-12 days to allow for flexibility in producing a sufficient number of cells in the final product.

3.1.1.1 Dose Escalation

Note: The paragraphs highlighted in grey are no longer applicable after the approval of Amendment Z.

Beginning with Amendment X, Cohort 13 has been completed with one patient enrolled with no DLTs observed and Cohort 14 was opened.

Initially 6 patients will be enrolled in Cohort 14. If less than or equal to one DLT is seen in the first 6 patients enrolled in Cohort 14, the cohort will be expanded to a total of 12 patients to further characterize the safety of the maximum tolerated dose.

With the approval of Amendment Y, if 2 or more DLTs are encountered in Cohort 14, Cohort 11 will re-open and will be expanded to a maximum of 12 patients provided that less than 2 in 12 patients have a DLT in this cohort. If 2 or more DLTs are observed in Cohort 11, we will re-evaluate our strategy.

With the approval of Amendment Z, if ≤ 2 of 6 patients experience a DLT in Cohort 11 then the cohort will be expanded to a maximum of 18 patients provided that fewer than 4 in 18 patients have a DLT. If 4 or more DLTs are observed in 18 patients in Cohort 11, we will de-escalate to Cohort 11D. The same DLT criteria as described below will be used to define the MTD for Cohort 11D. Note: With Amendment Z, the toxicities experienced by patient #4 in Cohort 11, will no longer meet the criteria for DLT.

If 9 or greater of 18 patients in Cohort 11 experiences a PR or CR lasting more than 3 months this will be declared the MTD regimen. If fewer than 9 patients experience a PR and/or CR, then Cohort 14 will be re-opened and up to a total of 18 patients may be enrolled. The same DLT criteria as described below will be used to define the MTD.

The number of cryopreserved anti-CD19-transduced T-cells transferred for each cohort will be:

Cohort	Treatment Regimen		
	<i>NMA Chemotherapy Regimen (as specified in protocol)</i>	<i>Cell dose (Determined prior to cryopreservation)</i>	<i>Days in Culture</i>
11-Completed	Cytosan 300mg/m ² & Fludarabine 30mg/m ²	2.0x10 ⁶ CAR positive cells/kg (\pm 20%)	6-12
11D- (De-escalation Cohort)	Cytosan 300mg/m ² & Fludarabine 30mg/m ²	1.0x10 ⁶ CAR positive cells/kg (\pm 20%)	6-12
12-Closed	Yes	6.0x10 ⁶ CAR positive cells/kg (\pm 20%)	6
13-Completed	Cytosan 500mg/m ² & Fludarabine 30mg/m ²	2.0x10 ⁶ CAR positive cells/kg (\pm 20%)	6
14-Completed	Cytosan 500mg/m ² & Fludarabine 30mg/m ²	2.0x10 ⁶ CAR positive cells/kg (\pm 20%)	6-12

The maximum tolerated cell dose is the highest dose at which ≤ 1 of 6 patients experienced a DLT or the highest dose level studied if DLTs are not observed at any of the dose levels.

Following administration of the cell product, neurological status will be closely monitored and urgently managed as described in Section 3.4.3.

In addition, we will be evaluating stored serum samples in an effort to identify the particular cytokines which may be contributing to these toxicities.

3.1.1.2 Safety Assessment

Cytokine (IFN-gamma levels) and toxicity data must be analyzed prior to dose de-escalation. A one-week safety assessment period will follow regimen completion between the first patient in each cohort before a second patient will be accrued in the cohort.

3.1.2 Definition of Dose-Limiting Toxicity (DLT)

Dose-limiting toxicity is defined as follows:

- Grade 4 neutropenia lasting longer than 21 days from the day of cell transfer
- Grade 4 thrombocytopenia lasting longer than 35 days from the day of cell transfer
- All grade 3 toxicities lasting more than 3 days (including grade 3 hypotension requiring the use of pressors) and all grade 4 toxicities with the exception of:
 - Myelosuppression (includes bleeding in the setting of platelet count less than 50,000 per mcl and documented bacterial infections in the setting of neutropenia), defined as lymphopenia, decreased hemoglobin, neutropenia and thrombocytopenia” unless neutropenia and thrombocytopenia meet the DLT definition described above.
 - Expected chemotherapy toxicities as defined in Section 11.
 - Grade 3 Fever
 - Immediate hypersensitivity reactions occurring within 2 hours of cell infusion (related to cell infusion) that are reversible to a grade 2 or less within 24 hours of cell administration with standard therapy.
 - Aphasia/dysphagia or confusion/cognitive disturbance which resolve to grade 1 or less within 2 weeks.

Note: Neurology consults and MRIs of the brain will be conducted on any subject experiencing any grade 3 or greater neurologic toxicity.

Dose Limiting Toxicities per Cohort as of 4/13/2015

Cohort	Treatment Regimen			
	<i>NMA Chemotherapy Regimen</i>	<i>Cell Dose</i>	<i># of Patients per Cohort</i>	<i># of DLTs per Cohort</i>
		<i>Fresh Cells</i> <i>10-25 Days in Culture</i>		
1	Cytosan 60mg/kg x 2 days & Fludarabine 25mg/m ² x 5 days	1.0x10 ⁹ – 1.0x10 ¹⁰ CAR transduced cells	8	3
6	Cytosan 60mg/kg x 2 days & Fludarabine 25mg/m ² x 5 days	0.5x10 ⁷ CAR positive cells/kg (± 20%)	2	2
8	Cytosan 60mg/kg x 2 days &	2.5x10 ⁶ CAR positive	5	2

	Fludarabine 25mg/m ² x 5 days	cells/kg (\pm 20%)		
9	Cytosan 30mg/kg x 2 days & Fludarabine 25mg/m ² x 5 days	1.0x10 ⁶ CAR positive cells/kg (\pm 20%)	6	2
10	Cytosan 300mg/m ² & Fludarabine 30mg/m ² x 3 days	1.0x10 ⁶ CAR positive cells/kg (\pm 20%)	7	None
		<i>Cryopreserved Cells 6-8 Days in Culture</i>		
11	Cytosan 300mg/m ² & Fludarabine 30mg/m ² x 3 days	2.0x10 ⁶ CAR positive cells/kg (\pm 20%)	4	1
12	Cytosan 300mg/m ² & Fludarabine 30mg/m ² x 3 days	6.0x10 ⁶ CAR positive cells/kg (\pm 20%)	1	1
13	Cytosan 500mg/m ² & Fludarabine 30mg/m ² x 3 days	2.0x10 ⁶ CAR positive cells/kg (\pm 20%)	2	1
14	Cytosan 500mg/m ² & Fludarabine 30mg/m ² x 3 days	2.0x10 ⁶ CAR positive cells/kg (\pm 20%)	2	2

3.2 PROTOCOL STOPPING RULES

Note: The paragraphs highlighted in grey are no longer applicable after the approval of Amendment S.

The study will be halted pending discussions with the FDA and IRB if the following conditions are met:

Prior to Amendment E

- If two DLTs occur in the first cohort of the Phase I portion of this study.

With approval of Amendment E (not applicable with approval of Amendment F):

- If two DLTs occur in the first 6 patients treated on this study.
- Two or more patients develop a grade 3 or greater toxicity at any point in the study not attributable to the chemotherapy preparative regimen (or circumstances unrelated to this study).
- If 1 of the first 3 patients (OR 2 of the first 6 patients, OR 3 of the first 9 patients, OR 4 of the first 12 patients) develop DLT due to autoimmune toxicity.
- Development of EBV lymphoma or polyclonal lymphoproliferative disease (PLPD) in an EBV negative subject on this or any other SB adoptive cell therapy study. Accrual of EBV negative subjects will be halted only.

With Approval of Amendments F, H, and J

As stated above, the study will be halted pending discussions with the FDA and IRB if the following conditions are met:

- If cohorts 2-7 or 9 cannot be expanded due to DLTs.

- During the expansion phase, if at any time during accrual, a cumulative total of 3 evaluable patients have experienced a DLT.
- Two or more patients develop a grade 3 or greater toxicity at any point in the study not attributable to the chemotherapy preparative (or circumstances unrelated to this study).
- If 1 of the first 3 patients (OR 2 of the first 6 patients, OR 3 of the first 9 patients, OR 4 of the first 12 patients) in cohorts 2-5 develop DLT due to autoimmune toxicity.
- Development of EBV lymphoma or polyclonal lymphoproliferative disease (PLPD) in an EBV negative subject on this or any other SB adoptive cell therapy study. Accrual of EBV negative subjects will be halted only.

With Approval of Amendment X

As stated above, the study will be halted pending discussions with the FDA and IRB if the following conditions are met:

- Two or more patients develop a grade 4 or greater toxicity at any point in the study not attributable to the chemotherapy preparative (or circumstances unrelated to this study).
NOTE: Toxicities excluded from the determination of DLTs are not included
- If 1 of the first 3 patients (OR 2 of the first 6 patients, OR 3 of the first 9 patients, OR 4 of the first 12 patients) in cohorts 11-14 develop DLT due to autoimmune toxicity.
- Development of EBV lymphoma or polyclonal lymphoproliferative disease (PLPD) in an EBV negative subject on this or any other SB adoptive cell therapy study. Accrual of EBV negative subjects will be halted only.
- If one or more treatment related deaths occur due to the cell infusion, we will promptly discuss this with the IRB and FDA.

3.3 DRUG ADMINISTRATION

Note: The following dose adjustments have been made over the course of the protocol:

- With approval of Amendment H, aldesleukin was removed.
- With the approval of Amendment M, the dose of cyclophosphamide was reduced from 60 mg/kg/day to 30 mg/kg/day.
- With the approval of Amendment P, the dose of cyclophosphamide was further reduced from 30 mg/kg/day x 2 days to 300 mg/m²/day x 3 days and the dose of fludarabine was reduced from 25mg/m²/day x 5 days to 30 mg/m² x 3 days – decreasing the total dose of cyclophosphamide by more than 50% and fludarabine by about 30%.
- With the approval of Amendment V, the dose of cyclophosphamide was increased from 300 mg/m²/day x 3 days to 500 mg/m²/day x 3 days.
- With the approval of Amendment Y, the dose of cyclophosphamide was reverted back to 300 mg/m²/day x 3 days.

3.3.1 Preparative Regimen with Cyclophosphamide and Fludarabine

Days -5 through -3

- 1.0L of 0.9% NaCl given at 500 mL/hour starting 2 hours prior to cyclophosphamide followed by:
- Cyclophosphamide 300mg/m² IV over 60 minutes followed by:
- Fludarabine 30mg/m² IV over 30 minutes followed by:
- An additional 1.0L of 0.9% NaCl given at 500 mL/hour.

Patients should be instructed to drink plenty of liquids during and for 24 hours following the chemotherapy [approximately 2 liters/24 hours]; hydration may be adjusted as clinically indicated.

3.3.2 Cell Infusion

Cells are delivered to the patient care unit by a staff member from the Tumor Immunology Cell Processing Laboratory. Prior to infusion, the cell product identity label is double-checked by two authorized staff (MD or RN), an identification of the product and documentation of administration are entered in the patient's chart, as is done for blood banking protocols. The cells are to be infused intravenously over 20-30 minutes via non-filtered tubing, gently agitating the bag during infusion to prevent cell clumping. Note: with approval of amendments F-H, the dose of cells administered will be on a CAR positive cells/kg basis.

Aldesleukin will not be administered with approval of Amendment H.

Note: With the approval of Amendment S, the dose of cells administered will be cryopreserved anti-CD19 CAR positive cells/kg ($\pm 20\%$).

Day 0 (2-4 days after the last dose of fludarabine)

- Cells will be thawed in the TIL lab and infused intravenously (i.v.) on the Patient Care Unit over 20 to 30 minutes.

Day 1-4 (Day 0 is the day of cell infusion): Beginning with Amendment P

- Filgrastim will be initiated at a dose of 300 micrograms daily for patients under 70 kg in weight and a dose of 480 micrograms daily for patients over 70 kg in weight only if the absolute neutrophil count is less than 650/microliter. Filgrastim will be discontinued as soon as the neutrophil count is 2000/microliter or higher

3.3.3 Study Calendar

Day	-9	-8	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4
Therapy														
Cyclophosphamide					X	X	X							
Fludarabine					X	X	X							
Cryopreserved Anti-CD19 CAR PBL ¹										X ¹				

Filgrastim ²											X	X	X	X
Allopurinol ⁶	X	X	X	X	X	X	X	X	X	X	X	X	X	X
TMP/SMX ³ 160mg/800mg (example)			X	X	X	X	X	X	X	X	X	X	X	X
Fluconazole ⁴ (400 mg po)										X	X	X	X	X
Valacyclovir po or Acyclovir IV ⁵										X	X	X	X	X

¹Two to four days after the last dose of fludarabine

²Filgrastim will be initiated at a dose of 300 micrograms daily for patients under 70 kg in weight and a dose of 480 micrograms daily for patients over 70 kg in weight only if the absolute neutrophil count is less than 650/microliter. Filgrastim will be discontinued as soon as the neutrophil count is 2000/microliter or higher.

³The TMP/SMX schedule should be adjusted to QD three times per week (Monday, Wednesday, Friday) and continue for at least six months and until CD4 > 200 X 2

⁴Continue until ANC > 1000/mm³

⁵Continue for at least six months and until CD4 > 200 X 2.

⁶For patients at high risk of developing Tumor Lysis Syndrome only.

3.4 ON-STUDY EVALUATION

Note: Refer to Section 5 for research evaluations.

3.4.1 Prior to Starting the Preparative Regimen

- Apheresis as indicated
- Within 14 days prior to starting the preparative regimen, patients will have a complete blood count, serum chemistries performed including electrolytes, BUN, creatinine, and liver function tests. If any results are beyond the criteria established for eligibility, the patient will not proceed until the abnormalities can be resolved.

3.4.2 During the Preparative Regimen (Daily)

- Complete Blood Count
- Chem 20 equivalent: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO² (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid)
- Urinalysis

3.4.3 After Cell Infusion

- Vital signs, including neurological checks, will be monitored hourly (\pm 15 minutes) for four hours and then routinely (every 4-6 hours)). If the patient has been febrile (Temp 38.3 or more) at any time in the preceding 24 hours, vital signs will be monitored strictly every 4 hours.

- Neurological evaluations: If the patient experiences a grade 3 or greater neurological toxicity the following evaluations will be performed:
 - Neurology Consult - urgent
 - MRI of the Brain
 - Lumbar puncture to evaluate transduced cells in the CSF if the platelet count is greater than 50,000/mm³. **Intrathecal dexamethasone 8mg will be administered at time of the LP, as clinically indicated.**
- Once total lymphocyte count is greater than 200/mm³, TBNK for peripheral blood CD4 count will be drawn weekly (while the patient is hospitalized). Please refer to Section 5 for additional post cell infusion evaluations.

3.4.4 During Hospitalization (Every 1-2 Days as Clinically Indicated)

- A review of systems and physical exam, including focused neurological examination
- CBC
- Chem 20 equivalent: Sodium (Na), Potassium (K), Chloride (Cl), Total CO² (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid
- Other tests will be performed as clinically indicated.

3.5 RETREATMENT

Prior to Amendment N

Patients will be evaluated 4 to 6 weeks after the initial treatment regimen (defined as the end of the last aldesleukin dose, with approval of Amendment H, defined as the end of cell infusion). If patients have a partial response to treatment and their disease subsequently progresses, they may be re-treated when progression based on the appropriate anti-malignancy response criteria (see Section 6.3) is documented after evaluation by Principal Investigator with the same schedule that they had been given safely (grade 3 toxicity due to cell infusion which is reversible within 24 hours with supportive measures may be retreated, but patients who develop grade 4 toxicity due to cell infusion will not be retreated.) Patients must continue to meet the original eligibility criteria to be considered for retreatment. Retreatment will consist of the non-myeloablative chemotherapy regimen, cell infusion, and aldesleukin. With the approval of Amendment H, aldesleukin will not be included in the retreatment regimen. Toxicity related to cyclophosphamide or fludarabine should be stable and resolved to less than grade 1 prior to re-treatment. Re-treatment benefits and risks will be carefully explained to the patient. Patients must be reconsented prior to retreatment. Re-treatment would begin 6 to 8 weeks after the last aldesleukin dose. A maximum of one retreatment course may occur.

Following Amendment S

Patients who experience disease progression following a confirmed complete or partial response to treatment or who have residual disease at the second or subsequent assessments following the initial treatment may be re-treated. Patients will be retreated at the currently enrolling dose level, but it will not count towards establishing the MTD. Patients who develop grade 3 toxicity due to

cell infusion which is reversible within 24 hours with supportive measures may be retreated. Patients who develop grade 4 toxicity due to cell infusion will not be retreated. Patients must continue to meet the original eligibility criteria and be negative for HAMA (Biolegend human anti-mouse IgG ELISA) to be considered for retreatment. Retreatment will consist of the non-myeloablative chemotherapy regimen followed by cryopreserved cell infusion. Toxicity related to cyclophosphamide or fludarabine should be stable and resolved to less than grade 1 prior to retreatment. Retreatment benefits and risks will be carefully explained to the patient. A maximum of one retreatment course may occur.

3.6 POST-STUDY EVALUATION (FOLLOW-UP)

- All patients will return to the NIH Clinical Center for evaluation 6 weeks (\pm 2 weeks) following the administration of the cell product.
- Patients discharged with grade 3 or greater significant adverse events should be evaluated by referring physician within 2 weeks of discharge.

3.6.1 Time-Period of Evaluations

Patients who experience stable disease, a partial response, or a complete response or have unresolved toxicities will be evaluated as noted below:

- Week 12 (\pm 2 weeks)
- Every 3 months (\pm 1 month) x3
- Every 6 months (\pm 1 month) x2
- As per PI discretion for subsequent years

Note: Patients may be seen more frequently as clinically indicated.

3.6.2 Scheduled Evaluations

At each evaluation, patients will undergo:

- Physical examination
- Chem 20 equivalent: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO² (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid), Complete blood count
- Thyroid panel as clinically indicated
- TBNK
- Toxicity assessment, including a review of systems
- CT of the chest, abdomen and pelvis and PET scan. This end of course evaluation will be used to determine response of the malignancy to treatment. If clinically indicated, other scans or x-rays may be performed, e.g. brain MRI, bone scan.
- Visual symptoms will be evaluated and if changes have occurred from baseline, i.e. changes in visual acuity, an ophthalmologic consult will be performed.

- A 5-liter apheresis may be performed at the first follow up visit. If the patient is unable to undergo apheresis, approximately 96 mL of blood may be obtained. Subsequently, 60 mL of blood will be obtained at follow up visits (approximately monthly) for at least 3 months. Peripheral blood mononuclear cells will be cryopreserved so that immunologic testing may be performed.
- Detection of RCR and persistence of CAR gene transduced cells (Section 5.3)
- Note: Long-term follow up of patients receiving gene transfer: Physical examinations will be performed and documented annually for 5 years following cell infusion to evaluate long-term safety. After 5 years, health status data will be obtained from surviving patients via telephone contact or mailed questionnaires. The long term follow up period for retroviral vectors is 15 years.
- Note: Patients who are unable or unwilling to return for follow up evaluations will be followed via phone or email contact. Patients may be asked to send laboratory, imaging and physician exam reports performed by their treating physician.

3.7 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF-STUDY CRITERIA

3.7.1 Criteria for Removal from Protocol Therapy

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

Patients will be taken off treatment (and followed until progression of disease) for the following:

- Completion of protocol therapy.
- Patient requests to be withdrawn from active therapy.
- Investigator discretion.
- Positive pregnancy test.
- Tumor lysis syndrome, defined as a 25% or greater increase in the pre-treatment values of serum phosphorous, potassium, and uric acid as well as a 25% or greater decrease in serum calcium in the setting of effective treatment of a patient with large tumor burden.

3.7.2 Off-Study Criteria

Patients will be taken off study for the following:

- Completed study follow-up period
- Participant requests to be withdrawn from study
- Progressive disease, unless the patient is eligible for a second treatment.
- Lost to follow-up
- Death

Note: Once a subject is taken off-study, no further data can be collected.

Note: Patients who are taken off-study for progressive disease or study closure may be followed on protocol 09-C-0161 (Follow-up Protocol for Subjects Previously Enrolled on NCI Surgery Branch Studies).

3.7.3 Off Protocol Therapy and Off-Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off protocol therapy and when a subject is taken off-study. A Participant Status Updates Form from the web site (<http://home.ccr.cancer.gov/intra/eligibility/welcome.htm>) main page must be completed and sent via encrypted email to: NCI Central Registration Office, ncicentralregistration-1@mail.nih.gov.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 INFECTION PROPHYLAXIS

Note: Other anti-infective agents may be substituted at the discretion of the treating investigator.

4.1.1 Pneumocystis Jirovecii Pneumonia

Patients will receive the fixed combination of trimethoprim and sulfamethoxazole [SMX] as double strength (DS) tab (DS tabs = TMP 160 mg/tab, and SMX 800 mg/tab) PO daily three times a week on non-consecutive days, beginning between days -5 and -8.

Pentamidine will be substituted for TMP/SMX-DS in patients with sulfa allergies. It will be administered aerosolized at 300 mg per nebulizer within one week of chemotherapy start date and monthly thereafter.

4.1.2 Herpes Virus Prophylaxis

Patients will receive valacyclovir orally at a dose of 500 mg daily the day after chemotherapy ends, or acyclovir, 250 mg/m² IV every 12 hours if the patient is not able to take medication by mouth. Reversible renal insufficiency has been reported with IV but not oral acyclovir.

Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs have been reported with higher doses of acyclovir. Should this occur, a dosage adjustment will be made or the drug will be discontinued. Acyclovir will not be used concomitantly with other nucleoside analogs which interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling.

Prophylaxis for pneumocystis and herpes will continue for 6 months post chemotherapy. If the CD4 count is less than 200 at 6 months post chemotherapy, prophylaxis will continue until the CD4 count is greater than 200 X 2.

4.1.3 Fungal Prophylaxis

Patients will start fluconazole 400 mg PO the day after chemotherapy concludes and continue until the absolute neutrophil count is greater than 1000/mm³. The drug may be given IV at a dose of 400 mg in 0.9% sodium chloride USP daily in patients unable to take it orally.

4.1.4 Empiric Antibiotics

Patients will start on broad-spectrum antibiotics, either a 3rd or 4th generation cephalosporin or a quinolone for fever of 38.3°C once or two temperatures of 38.0°C or above at least one hour

apart, AND an ANC $<500/\text{mm}^3$. Infectious disease consultation will be obtained for all patients with unexplained fever or any infectious complications.

4.1.5 Prophylaxis and Treatment of Tumor Lysis Syndrome

Subjects deemed to be at high risk of tumor lysis syndrome should begin allopurinol at a dose of 200 mg PO every 8 hours. This should be started 2 days prior to the first dose of cyclophosphamide and continued until disease burden is reduced (e.g. peripheral blasts clear) or it is apparent that no tumor lysis has developed after 1 week of treatment.

4.2 BLOOD PRODUCT SUPPORT

Using daily CBCs as a guide, the patient will receive platelets and packed red blood cells (PRBCs) as needed. Attempts will be made to keep Hb $> 8.0 \text{ gm/dL}$, and plts $> 10,000/\text{mm}^3$. All blood products with the exception of the stem cell product will be irradiated. Leukocyte filters will be utilized for all blood and platelet transfusions to decrease sensitization to transfused WBCs and decrease the risk of CMV infection.

4.3 OTHER CONCOMITANT MEDICATIONS TO CONTROL SIDE EFFECTS

Concomitant medications to control side effects of therapy will be given. Meperidine (25-50 mg) will be given intravenously if severe chilling develops. Other supportive therapy will be given as required and may include acetaminophen (650 mg every 4 hours), indomethacin (50-75 mg every 6 hours) and ranitidine (150 mg every 12 hours). If patients require steroid therapy, they will be taken off treatment. Patients who require transfusions will receive irradiated blood products. Ondansetron 0.15 mg/kg/dose IV every 8 hours will be administered for nausea and vomiting. Additional antiemetics will be administered as needed for nausea and vomiting uncontrolled by ondansetron. Antibiotic coverage for central venous catheters may be provided at the discretion of the investigator.

5 BIOSPECIMEN COLLECTION

Blood and tissue are tracked at the patient level and can be linked to all protocols on which the patient has been enrolled. Samples will be used to support the specific objectives listed in the treatment protocol(s), e.g., immunologic monitoring, cytokine levels, persistence, as well as to support long term research efforts within the Surgery Branch and with collaborators as specified in protocol 03-C-0277.

5.1 RESEARCH EVALUATIONS

The amount of blood that may be drawn from adult patients for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period.

5.1.1 Prior to Chemotherapy Administration

- 5 CPT tubes (8 mL each)
- 1 SST tube (8 mL)
- SST tube (4 mL) daily; starting day of chemotherapy

5.1.2 Prior to Cell Infusion (1-8 mL SST)

- Blood samples for analysis for detection of RCR by PCR.

- Blood samples for analysis of the cytokines IL-6, TNF- α , IFN- γ and GM-CSF.

5.1.3 Post-Cell Infusion Evaluations

Once total lymphocyte count is greater than 200/mm³, the following samples will be drawn and sent to the TIL lab on Monday, Wednesday, and Friday x5 days, then weekly (while the patient is hospitalized):

- 5 CPT tubes (8 mL each)
- 1 SST tube (8 mL)

5.2 IMMUNOLOGICAL TESTING

- Apheresis may be performed, prior to and 4-6 weeks after the treatment. At other time points, patient peripheral blood lymphocytes (PBL) will be obtained from whole blood by purification using centrifugation on a Ficoll cushion.
- Lymphocytes will be tested directly and following in vitro culture. Direct immunological monitoring will consist of quantifying CD3+ T-cells that express the anti-CD19 CAR by FACS analysis using a goat-anti-mouse antibody as shown in [Figure 2](#). *Ex vivo* immunological assays will consist of cytokine release by bulk PBL, intracellular cytokine staining as shown in [Figure 6](#), and by other experimental studies such as cytotoxicity if sufficient cells are available. If cell numbers are limiting, preference will be given to the direct analysis of immunological activity. Immunological assays will be standardized by the inclusion of 1) pre-infusion PBMC and 2) an aliquot of the engineered PBL cryopreserved at the time of infusion. In general, differences of 2- to 3-fold in these assays are indicative of true biologic differences.

Note: The collection and analysis of research labs will be monitored by the TIL lab and not by the CCR contractor.

5.3 MONITORING GENE THERAPY TRIALS: PERSISTENCE AND RCR

- Immunological monitoring by using either anti-mouse Fab FACS staining to detect CARs on the surface of T-cells or a quantitative real-time PCR assay will be used to quantitate persistence of T-cells in the blood. Persistence will be determined at about 1 week, 4 weeks (± 2 weeks), 3 months (± 1 month), 6 months (± 1 month), and 12 months (± 1 month) after cell infusion.
- All patients will be co-enrolled on protocol 09-C-0161. Patients' blood samples will be obtained and undergo analysis for detection of RCR by PCR prior to cell infusion and RCR PCR will be performed at 3 and 6 months, and at one year post-cell administration. Blood samples will be archived annually thereafter if all previous testing has been negative with a brief clinical history. If a patient dies or develops neoplasms during this trial, efforts will be made to assay a biopsy sample for RCR. If any post-treatment samples are positive, further analysis of the RCR and more extensive patient follow-up will be undertaken, in consultation with the FDA. RCR PCR assays detect the GaLV envelop gene and are performed under contract by the Indiana University Vector Production Facility. The results of these tests are maintained by the contractor performing the RCR tests and by the Surgery Branch research team.

5.4 SAMPLE STORAGE, TRACKING, AND DISPOSITION

Blood and tissue collected during the course of this study will follow the Cell Tracking and Labeling System established by the Tumor Immunology Cell Processing Laboratory. The Cell Tracking and Labeling System is designed to unambiguously ensure that patient/data verification is consistent. The patients' cell samples (blood or tissue) are tracked by distinct identification labels that include a unique patient identifier and date of specimen collection. Cryopreserved blood and tissue samples also bear the date the sample was frozen. All cryopreserved samples are tracked for freezer location and storage criteria. All samples are stored in monitored freezers/refrigerators in 3NW Surgery Branch Laboratories at specified temperatures with alarm systems in place. Serum samples will be sent to the Clinical Pharmacology Program (CPP) for storage. Samples will be barcoded and stored on site or offsite at NCI Frederick Central Repository Services in Frederick, MD. Data is entered and stored securely in the Patient Sample Data Management System (PSDMS) utilized by the CPP, and data will be updated to the Surgery Branch central computer database weekly. All samples (blood or tissue) are entered into a central computer database with identification and storage location, and this database is backed up every night.

At the conclusion of this protocol, if additional studies are to be performed on any samples obtained during the conduct of this trial, a Request to Conduct Research for Stored Human Samples Specimens, or Data Collected in a Terminated IRB Protocol will be submitted. Otherwise, specimens will be disposed of in accordance with the environmental protection laws, regulations and guidelines of the Federal Government and the State of Maryland.

Any loss or unintentional destruction of the samples will be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

Note: Blood and tissue collected during the course of this study will be stored, tracked and disposed of as specified in protocol 03-C-0277.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system (C3D) and ensuring data accuracy, consistency and timeliness. The Principal Investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant. Data will be entered into the NCI CCR C3D database.

All adverse events (AEs), including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Patients will be followed for AEs until 30 days following the last dose of study therapy or until off-study, whichever comes first.

An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study

- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

All AEs must be recorded on the AE case report form unless otherwise noted below in Section **6.1.1**.

End of study procedures: Data will be stored according to HHS and FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

6.1.1 Routine Adverse Event Recording

All adverse events will be recorded in the patient's medical record. Following registration through 30 days after the cell infusion adverse events will be reviewed by the research nurse and Principal Investigator and captured in the C3D database. All events occurring during the treatment phase of the study will be followed until resolution or stabilization. During the follow up period, only grade 3 and 4 and unexpected grade 2 events that are related to the treatment will be captured in C3D.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

De-identified human data generated for use in future and ongoing research will be shared through a NIH-funded or approved repository (ClinicalTrials.gov) and BTRIS. At the completion of data analysis, data will be submitted to ClinicalTrials.gov either before publication or at the time of publication or shortly thereafter. Data may also be used to support long term research efforts within the Surgery Branch and de-identified data may also be shared with collaborators as specified in protocol 03-C-0277.

6.2.2 Genomic Data Sharing Plan

All genomic analysis that requires compliance with the GDS policy is being performed on protocol 03-C-0277.

6.3 RESPONSE CRITERIA

6.3.1 Anti-Malignancy Response Criteria

6.3.1.1 Response Criteria for Lymphoma

Note: All responses must last for at least 4 weeks after treatment (Cheson et al. Revised Response Criteria for Malignant Lymphoma, Journal of Clinical Oncology 2007 ⁴⁸).

- Complete Remission (CR): CR requires all of the following:
 1. Complete disappearance of all detectable clinical evidence of disease and disease-related symptoms if present before therapy.
 2. Typically, FDG-avid lymphoma (large cell, mantle cell and follicular lymphomas are all typically FDG-avid): in patients with no pretreatment PET scan or when the PET scan was positive before therapy, a post-treatment residual mass of any size is permitted as long as it is PET negative.
 3. Variably FDG-avid lymphomas/FDG avidity unknown: in patients without a pretreatment PET scan, or if a pretreatment PET scan was negative, all lymph nodes and nodal masses must have regressed to normal size (≤ 1.5 cm in greatest diameter if > 1.5 cm before therapy). Previously involved nodes that were 1.1 to 1.5 cm in their long axis and more than 1 cm in their short axis before treatment must have decreased to ≤ 1.0 cm in their short axis after treatment.
 4. The spleen and/or liver, if considered to be enlarged before therapy on basis of physical exam or CT scan, must should be normal size on CT scan and not be palpable on physical examination and nodules thought to represent lymphoma must no longer be present.
 5. A bone marrow aspirate and biopsy is performed only when the patient had bone marrow involvement with lymphoma prior to therapy or if new abnormalities in the peripheral blood counts or blood smear cause clinical suspicion of bone marrow involvement with lymphoma after treatment. The bone marrow aspirate and biopsy must show no evidence of disease by morphology or if indeterminate by morphology it must be negative by immunohistochemistry. The biopsy core sample must be a minimum of 20 mm in length.
- Partial Remission (PR): PR requires all of the following:
 1. $\geq 50\%$ decrease in sum of the product of the diameters (SPD) of up to 6 of the largest dominant nodes or nodal masses. Dominant nodes or nodal masses should be clearly measurable in at least 2 perpendicular dimensions, should be from different regions of the body if possible and should include mediastinal and retroperitoneal nodes if possible.
 2. No increase in size of nodes, liver or spleen and no new sites of disease.
 3. If multiple splenic and hepatic nodules are present, they must regress by $\geq 50\%$ in the SPD. There must be a $\geq 50\%$ decrease in the greatest transverse diameter for single nodules.
 4. Bone marrow is irrelevant for determination of a PR. If patient has persistent bone marrow involvement and otherwise meets criteria for CR the patient will be considered a PR.
 5. Typically, FDG-avid lymphoma: for patients with no pretreatment PET scan or if the PET scan was positive before therapy, the post-treatment PET scan should be positive in at least one previously involved site. Note: in patients with follicular lymphoma or

mantle-cell lymphoma, a PET scan is only indicated in patients with one or at most two residual masses that have regressed by 50% on CT scan.

- Progressive Disease (PD): Defined by at least one of the following:
 1. $\geq 50\%$ increase from nadir in the sum of the products of at least two lymph nodes, or if a single node is involved at least a 50% increase in the product of the diameters of this one node.
 2. Appearance of a new lesion greater than 1.5 cm in any axis even if other lesions are decreasing in size
 3. Greater than or equal to a 50% increase in size of splenic or hepatic nodules
 4. At least a 50% increase in the longest diameter of any single previously identified node more than 1 cm in its short axis.
 5. Lesions should be PET positive in typically FDG-avid lymphomas unless the lesion is too small to be detected by PET (<1.5 cm in its long axis by CT)
- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD. PET should be positive in typically FDG-avid lymphomas.

Flow cytometric, molecular or cytogenetic studies will not be used to determine response.

6.4 PERSISTENCE CRITERIA FOR ANTI-CD19 CAR TRANSDUCED T-CELLS

The absolute number of persisting anti-CD19-CAR-transduced T-cells will be calculated by multiplying the absolute peripheral blood lymphocyte (PBL) count by the percentage of PBL that express both CD3 and the anti-CD19 CAR. The percentage of PBL that express both CD3 and the anti-CD19 CAR will be determined by a flow cytometry assay that involves staining with anti-CD3 and a polyclonal goat anti-mouse antibody product that is specific for mouse IgG F(ab')₂ antibody fragments (Jackson ImmunoResearch).

This analysis will be performed at about 2 weeks, 4 weeks (± 2 weeks), 3 months (± 1 month), and 6 months (± 1 month) after cell infusion. The 4-week time point is the only primary analysis that will be used for the primary evaluation of the number of CD3⁺ cells that express the anti-CD19 CAR.

6.5 TOXICITY CRITERIA

This study will utilize the CTCAE version 3.0 for toxicity and adverse event reporting. A copy of the CTCAE v3.0 can be downloaded from the CTEP home page (<http://ctep.cancer.gov>).

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event

Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research.

7.1.2 Suspected Adverse Reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, ‘reasonable possibility’ means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.1.3 Unexpected Adverse Reaction

An adverse event or suspected adverse reaction is considered “unexpected” if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

"Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.5 Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.1.6 Disability

A substantial disruption of a person’s ability to conduct normal life functions.

7.1.7 Life-Threatening Adverse Drug Experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

7.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB-approved research protocol.

7.1.9 Non-Compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

1. Is unexpected in terms of nature, severity, or frequency in relation to
 - a. the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and
 - b. the characteristics of the subject population being studied; **AND**
2. Is related or possibly related to participation in the research; **AND**
3. Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 IRB AND CLINICAL DIRECTOR (CD) REPORTING

7.2.1 IRB and NCI CD Expedited Reporting of Unanticipated Problems, and Deaths

The Protocol PI will report on the NIH Problem Form to the IRB and the NCI Clinical Director:

- All deaths, except deaths due to progressive disease.
- All protocol deviations
- All unanticipated problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance.
3. A tabular summary of the following adverse events:
 - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
 - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;

- All Grade 5 events regardless of attribution;
- All serious events regardless of attribution.

Note: Grade 1 events are not required to be reported.

To ensure safety using this treatment, the Surgery Branch will review safety data on all protocols at the time of continuing review. Data will be presented for both the recent period and for the entire length of time the protocol has been open. The toxicity data for review will include all toxicities captured on the protocol and will be presented in individual tables as follows:

- all toxicities attributed to the cells
- all incidences of intubation including the duration of and reason for intubation

7.2.3 IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the IRB.

7.3 IND SPONSOR REPORTING CRITERIA

From the time the subject receives the investigational agent/intervention to 30 days following the last dose of study therapy, the investigator must immediately report to the sponsor, using the mandatory MedWatch Form FDA 3500A or equivalent, any serious adverse event, whether or not considered drug-related, including those listed in the protocol or Investigator's Brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event. For serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention, only those events that have an attribution of at least possibly related to the agent/intervention will be reported.

- Death (except death due to progressive disease) must be reported via email within 24 hours. A complete report must be submitted within one business day.
- Other serious adverse events as well as deaths due to progressive disease must be reported within one business day.

Events will be submitted to the Center for Cancer Research (CCR) at: CCRsafety@mail.nih.gov and to the CCR PI and study coordinator.

7.3.1 Reporting Pregnancy

7.3.1.1 Maternal Exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately and the pregnancy reported to the Sponsor. The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agents (s) should be documented in box B5 of the MedWatch form "Describe Event or Problem".

Pregnancy itself is not regarded as an SAE. However, as patients who become pregnant on study risk intrauterine exposure of the fetus to agents which may be teratogenic, the CCR is requesting that pregnancy should be reported in an expedited manner as Grade 3 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy)" under the Pregnancy, puerperium and perinatal conditions SOC.

Congenital abnormalities or birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

If any pregnancy occurs in the course of the study, then the investigator should inform the Sponsor within 1 day, i.e., immediately, but no later than 24 hours of when he or she becomes aware of it.

The designated Sponsor representative will work with the investigator to ensure that all relevant information is provided to the Sponsor within 1 to 5 calendar days for SAEs and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

7.3.1.2 Paternal Exposure

Male patients should refrain from fathering a child or donating sperm during the study and for (120 days) after the cell infusion.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until (120 days) after the last dose should, if possible, be followed up and documented.

7.4 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

7.4.1 Serious Adverse Event Reports to IBC

The Principal Investigator (or delegate) will notify IBC of any unexpected fatal or life-threatening experience associated with the use of anti-CD19 CAR-transduced PBL as soon as possible but in no event later than 7 calendar days of initial receipt of the information. Serious adverse events that are unexpected and associated with the use of the anti-CD19 CAR-transduced PBL, but are not fatal or life-threatening, must be reported to the NIH IBC as soon as possible, but not later than 15 calendar days after the investigator's initial receipt of the information. Adverse events may be reported by using the FDA Form 3500a.

7.4.2 Annual Reports to IBC

Within 60 days after the one-year anniversary of the date on which the IBC approved the initial protocol, and after each subsequent anniversary until the trial is completed, the Principal Investigator (or delegate) shall submit the information described below. Alternatively, the IRB continuing review can be sent to the IBC in lieu of a separate report. Please include the IBC protocol number on the report.

7.4.2.1 Clinical Trial Information

A brief summary of the status of each trial in progress or completed during the previous year. The summary is required to include the following information for each trial:

- the title and purpose of the trial
- clinical site
- the Principal Investigator

- clinical protocol identifiers
- participant population (such as disease indication and general age group, e.g., adult or pediatric);
- the total number of participants planned for inclusion in the trial; the number entered into the trial to date whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons
- the status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed,
- if the trial has been completed, a brief description of any study results.

7.4.2.2 Progress Report and Data Analysis

Information obtained during the previous year's clinical and non-clinical investigations, including:

- a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system
- a summary of all serious adverse events submitted during the past year
- a summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications
- if any deaths have occurred, the number of participants who died during participation in the investigation and causes of death
- a brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

7.5 DATA AND SAFETY MONITORING PLAN

Careful evaluation to ascertain the toxicity, immunologic effects and anti-malignancy efficacy of cell infusions will be performed. Due to the nature of these studies, it is possible that expansion of specific T-cell clones will be observed as tumor reactive T-cell proliferate in response to tumor antigens. Therefore, care will be taken to track T-cell persistence both immunologically and molecularly according to plan specified in Section 6.4.

The Principal Investigator will review all serious adverse events and will monitor the data and toxicities to identify trends monthly. The Principal Investigator will be responsible for revising the protocol as needed to maintain safety.

7.5.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis when patients are being actively treated on the trial to discuss each patient. Decisions about enrollment will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the Principal Investigator. Adverse events will be reported as required above. Any safety concerns, new information that might

affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS.

The Principal Investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The Principal Investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.5.2 Sponsor Monitoring Plan

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary study endpoints; adherence to the protocol, regulations, and SOPs; and human subject's protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring
- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by a CCR contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

7.5.3 Safety Monitoring Committee (SMC)

This protocol will require oversight from the Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period. Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal Investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

8 STATISTICAL CONSIDERATIONS

Note: Paragraphs highlighted in grey below are no longer applicable after the approval of Amendment V.

The statistical section has been revised numerous times. Please refer to the bolded section below for the current statistical analysis for Amendment J. The primary endpoints of this trial are to determine safety and to determine whether lymphodepletion with fludarabine and cyclophosphamide enhances persistence of adoptively transferred, anti-CD19-CAR-transduced T-cells. The secondary endpoint is to determine the anti-malignancy effect of the T-cells

transduced with an anti-CD19 CAR in the patients that do not receive fludarabine and cyclophosphamide.

The initial portion of this protocol will be a phase I dose escalation design with three cohorts of a minimum of 3 patients per cohort. The number of anti-CD19-transduced T-cells transferred for each cohort will be:

Cohort 1	between	1.0×10^9 and 1.0×10^{10}
Cohort 2	greater than	1.0×10^{10} up to 3.0×10^{10}
Cohort 3	greater than	3.0×10^{10} up to 1.0×10^{11}

Patients will be enrolled sequentially, therefore enrollment will not proceed to a higher dose level until all patients have been treated in the prior cohort. If sufficient cells cannot be grown to meet the criteria for the assigned cohort, the patient will be enrolled in the appropriate cohort for the number of cells infused. Should a single patient experience a dose limiting toxicity at a particular dose level, three more patients would be treated at that dose to confirm that no greater than 1/6 patients have a DLT prior to proceeding to the next higher level. If a level with 2 or more DLTs in 3-6 patients has been identified, three additional patients will be accrued at the next-lowest dose for a total of 6, in order to further characterize the safety of the maximum tolerated dose prior to starting the phase II portion of the trial.

In phase II of the trial patients will be randomized to two treatment arms. Patients assigned to one treatment arm will receive fludarabine and cyclophosphamide lymphodepleting chemotherapy prior to receiving the engineered PBL cells while patients assigned to the other treatment arm will not receive chemotherapy. One to four days after completion of chemotherapy, anti-CD19 CAR-transduced T-cells will be infused. After cell infusion, all patients will receive IV aldesleukin therapy (720,000 IU/kg every 8 hours for a maximum of 15 doses). The randomization performed during phase II of this trial will be stratified so that each arm will receive an equal number of patients with a total CD3 count greater than or equal to 500 cells/microliter, and each arm will receive an equal number of patients with a CD3 count less than 500 cells/microliter. Randomization will also be stratified so that an equal number of patients with circulating malignant cells (leukemia) will be entered on each arm of the phase II part of the trial. For this trial, a patient with circulating malignant cells (leukemia) will be defined as a patient with a pre-treatment peripheral blood lymphocyte count of greater than 4000 lymphocytes per microliter.

The degree of persistence of anti-CD19-CAR-transduced T-cells will be evaluated by a quantitative measure in all patients, and will be compared directly between the two arms in order to determine if the use of fludarabine and cyclophosphamide lymphodepleting chemotherapy prior to receiving the engineered PBL cells will enhance persistence. Persistence will be determined as described in Section 5.3. The 4-week time point will be used for the primary evaluation of percentage of CD3⁺ cells that express the anti-CD19 CAR. With 17 patients in each of the two randomized arms, there will be 80% power to identify a difference in the mean persistence parameter between the two arms which will be equal to one standard deviation of the values within each arm (effect size 1.0), using a 0.05 two-tailed two-sample t-test. In a previous trial of TCR gene transfer, in patients that received fludarabine and cyclophosphamide for lymphodepletion, 9-56% of transduced cells persisted at 1 to 4 weeks post-infusion. We anticipate that the persistence of cells will be decreased without the lymphodepleting

chemotherapy. In practice, if the persistence measure is not normally distributed in both arms ($p < 0.05$ by a Shapiro-Wilks test in either arm) then a Wilcoxon rank sum test will be used.

Anti-malignancy effects will be measured by clinical response as noted in Section 6.3, and will be reported using 95% confidence intervals, separately for CLL and for lymphoma. As there are no requirements for a particular number of each type of disease, the results will be interpreted cautiously in the context of a pilot study which may be used to guide determination of parameters for study in future protocols if warranted.

In order to complete enrollment to this protocol, up to 18 patients may be needed for the phase I portion of the trial, and an additional 34 for the phase II portion. Thus, at an accrual rate of 2 patients per month, up to 2.5 years may be required to enroll up to 52 patients onto this trial.

With Amendment E (closed with approval of Amendment F), the trial is being refocused to evaluate the safety and toxicity of the regimen studied at dose level 1, between 1.0×10^9 and 1.0×10^{10} cells. This has been selected as the dose to use in all patients on this study as a result of the experience reported to date with this regimen. Under this Amendment, the study will initially enroll up to 6 evaluable patients who will receive this level of cells (4 patients have already been treated), and if no more than 1 of the 6 has a DLT, then accrual will continue up to a total of 18 evaluable patients, subject to not exceeding 3 patients in total with a DLT. If at any time during accrual, a cumulative total of 3 evaluable patients have experienced a DLT, then no further patients will be enrolled. The upper one sided 90% confidence interval bound on 3/18 is 0.334, which is marginally tolerable, while the upper one sided 90% confidence interval bound on 4/18 is 0.396. At earlier accrual points the upper 90% confidence interval bounds are higher: 3/15 has a bound of 0.393 and 4/15 has an upper 90% confidence interval bound of 0.464. Thus, 3/18 with a DLT is consistent with approximately 1/3 or fewer patients having a DLT, while having 3 patients with DLT occur at an earlier point, or having >3 patients with DLT is consistent with $>1/3$ of patients having a DLT. These latter two conditions would be considered consistent with excessive toxicity.

With approval of Amendment F, the trial is being refocused to evaluate the safety and toxicity of the regimen studied at lower escalating dose levels since 3 DLTs were observed at dose level 1 (between 1.0×10^9 and 1.0×10^{10} cells). For Cohort 2, 3 patients will be treated with NMA, 0.5×10^7 CAR positive cells/kg ($\pm 20\%$) and high dose aldesleukin (720,000 IU/kg). This has been selected as the starting dose due to PK modeling of interferon gamma levels and toxicity data from the first 8 patients treated on this study. If one DLT is observed in a patient treated in Cohort 2, after analysis of the IFN-gamma cytokine data, 3 additional patients will be treated at this dose level (0.5×10^7 CAR positive cells/kg ($\pm 20\%$) after receiving NMA followed by a reduced dose of aldesleukin (72,000 IU/kg every 8 hours for a maximum of 15 doses) (Cohort 3). This 10-fold reduction of aldesleukin was previously investigated in a study of patients with renal cancer. The incidence of grade 3 and 4 aldesleukin related toxicities was less in patients treated with 72,000 IU/kg every 8 hours than those treated with the higher dose of 720,000 IU/kg aldesleukin, every 8 hours (Yang, J.C., et al., J Clin Oncol. 2003, 21(16):3127-32).

If no DLTs are observed in the 3 patients in Cohort 2, after analysis of the IFN-gamma cytokine data, 3 additional patients will be treated with NMA, 1.0×10^7 CAR positive cells/kg ($\pm 20\%$) high dose aldesleukin (720,000 IU/kg) (Cohort 4). If one DLT is observed in a patient treated in Cohort 4, 3 additional patients will be treated at this dose level (1.0×10^7 CAR positive cells/kg (\pm

20%) after receiving NMA followed by a reduced dose of aldesleukin (72,000 IU/kg every 8 hours for a maximum of 15 doses) (Cohort 5).

Cohort	Treatment Regimen		
	<i>NMA Chemotherapy Regimen (as specified in protocol)</i>	<i>Cell Dose</i>	<i>IL-2 Dose</i>
1 (Closed with Amendment F)	Yes	between 1.0×10^9 and 1.0×10^{10} cells	High dose (720,000 IU/kg)
2	Yes	0.5×10^7 CAR positive cells/kg ($\pm 20\%$)	High dose (720,000 IU/kg)
3 ¹	Yes	0.5×10^7 CAR positive cells/kg ($\pm 20\%$)	Low dose (72,000 IU/kg)
4	Yes	1.0×10^7 CAR positive cells/kg ($\pm 20\%$)	High dose (720,000 IU/kg)
5 ²	Yes	1.0×10^7 CAR positive cells/kg ($\pm 20\%$)	Low dose (72,000 IU/kg)

¹ Cohort 3 will be skipped if there are no DLTs in Cohort 2.

² Cohort 5 will be skipped if there are no DLTs in Cohort 4.

The highest dose cohort evaluated with no DLTs in 3 patients (either 3, 4 or 5) will be expanded to a total of 18 evaluable patients, subject to not exceeding 3 patients in total with a DLT. If at any time during accrual, a cumulative total of 3 evaluable patients have experienced a DLT, then no further patients will be enrolled. The upper one sided 90% confidence interval bound on 3/18 is 0.334, which is marginally tolerable, while the upper one sided 90% confidence interval bound on 4/18 is 0.396. At earlier accrual points the upper 90% confidence interval bounds are higher: 3/15 has a bound of 0.393 and 4/15 has an upper 90% confidence interval bound of 0.464. Thus, 3/18 with a DLT is consistent with approximately 1/3 or fewer patients having a DLT, while having 3 patients with DLT occur at an earlier point, or having >3 patients with DLT is consistent with >1/3 of patients having a DLT. These latter two conditions would be considered consistent with excessive toxicity.

Accrual will be halted to the study if none of the cohorts can be expanded due to DLTs.

At the conclusion of the study, toxicities will be tabulated according to worst grade per patient of each type of toxicity, and the fraction experiencing a DLT will be reported, along with appropriate confidence intervals.

In order to complete enrollment to this protocol, up to 35 patients may be needed in total (including the 8 patients already accrued to this study, the 9 patients for the dose escalation under Amendment F, the 15 additional patients included in the expanded cohort described above, and 3 additional patients in case patients need to be replaced for any reason. Thus, at an accrual rate of 6-8 patients per year, up to 4 years may be required to enroll up to 35 patients onto this trial.

Following approval of Amendment H, only two dose cohorts will be open for accrual: cohorts 6 and 7. For Cohort 6, 3 patients will be treated with NMA, and 0.5×10^7 CAR positive cells/kg ($\pm 20\%$). If one DLT is observed in a patient treated in Cohort 6, after analysis of the IFN-gamma cytokine data, accrual will be halted pending discussions with the FDA and IRB. If no DLTs are observed in the 3 patients in Cohort 6, after analysis of the IFN-gamma cytokine data, 3 additional patients will be treated with NMA, and 1.0×10^7 CAR positive cells/kg ($\pm 20\%$) in Cohort 7. If one DLT is observed in a patient treated in Cohort 7, 3 additional patients will be treated in Cohort 6. If 1 of 6 patients in Cohort 6 experiences a DLT, after analysis of the IFN-gamma cytokine data, accrual will be halted pending discussions with the FDA and IRB.

The highest dose cohort evaluated with no DLTs in 3 or more patients will be expanded to a total of 18 evaluable patients, subject to not exceeding 3 patients in total with a DLT.

Accrual will be halted to the study if neither cohorts 6 nor 7 can be expanded due to DLTs.

In November 2011, the first patient in Cohort 6 experienced a DLT of grade 3 confusion (possible aphasia), and grade 3 neuropathy (facial droop) possibly related to the cell therapy. The serum IFN-gamma levels were low in this patient (range 9-44 pg/mL) following treatment with cells. These events were discussed with the FDA, and the FDA indicated that we can treat an additional patient in Cohort 6 (with approval of Amendment I). If this additional patient does not experience at DLT, Cohort 6 will be expanded to a total of 6 patients. If any additional patients treated in Cohort 6 experiences a DLT, after analysis of the IFN-gamma cytokine data, accrual will be halted pending discussions with the FDA and IRB. If no additional DLTs are observed in the 6 patients in Cohort 6, after analysis of the IFN-gamma cytokine data, 3 patients will be treated with NMA, and 1.0×10^7 CAR positive cells/kg ($\pm 20\%$) in Cohort 7. If one DLT is observed in a patient treated in Cohort 7, 3 additional patients will be accrued to Cohort 7. If 2 DLTs occur in 6 or less patients in Cohort 7, no additional patients will be accrued at this dose level. The highest dose cohort evaluated with no DLTs in 3 or more patients, or 1 DLT in 6 patients will be expanded to a total of 18 evaluable patients, subject to not exceeding 3 patients in total with a DLT.

In February 2012, the second patient in Cohort 6 experienced DLTs. The SAEs consisted of grade 3 hypotension and grade 3 confusion (possibly aphasia) related to the cell therapy. These toxicities have resolved and the patient has been discharged. After discussions with the FDA, cohorts 6 and 7 were closed and two new dose de-escalation cohorts were opened with approval of Amendment J as follows:

Cohort	Treatment Regimen		
	<i>NMA Chemotherapy Regimen (as specified in protocol)</i>	<i>Cell Dose</i>	<i>IL-2 Dose</i>
8 (De-escalation Dose 1)	Yes	2.5×10^6 CAR positive cells/kg ($\pm 20\%$)	None
9 (De-escalation Dose 2)	Yes	1.0×10^6 CAR positive cells/kg ($\pm 20\%$)	None

Cohort 8 will initially accrue 3 patients. If no DLTs are observed in these 3 patients, three additional patients will be accrued. If DLTs are observed in ≤ 1 patient out of 6 patients treated,

this cohort will be expanded as described below. If a DLT is observed in one of the first 3 patients, after analysis of the cytokine data, 3 additional patients can be accrued to this cohort. If more than 1 out of 6 patients in this cohort experience a DLT, this cohort will be closed, and patients will be accrued to Cohort 9. Cohort 9 will proceed in a similar fashion as Cohort 8. If more than 1 out of 6 patients in this cohort experience a DLT, this cohort will be closed. Subsequent dose reductions will be discussed with the FDA prior to initiation.

The highest dose cohort evaluated with DLTs in ≤ 1 patient in 6 patients treated will be expanded to a total of 18 evaluable patients, subject to not exceeding 3 patients in total with a DLT. If at any time during accrual, a cumulative total of 3 evaluable patients have experienced a DLT, then no further patients will be enrolled. The upper one sided 90% confidence interval bound on 3/18 is 0.334, which is marginally tolerable, while the upper one sided 90% confidence interval bound on 4/18 is 0.396. At earlier accrual points the upper 90% confidence interval bounds are higher: 3/15 has a bound of 0.393 and 4/15 has an upper 90% confidence interval bound of 0.464. Thus, 3/18 with a DLT is consistent with approximately 1/3 or fewer patients having a DLT, while having 3 patients with DLT occur at an earlier point, or having >3 patients with DLT is consistent with $>1/3$ of patients having a DLT. These latter two conditions would be considered consistent with excessive toxicity.

After discussions with the FDA, Cohort 8 was closed with Amendment M and patients will be lymphodepleted with fludarabine and a reduced dose of cyclophosphamide – 30mg/kg/day for 2 days and will receive 1.0×10^6 CAR positive cells/kg ($\pm 20\%$) in Cohort 9.

Cohort 9 will initially accrue 3 patients. If no DLTs are observed in these 3 patients, three additional patients will be accrued. If DLTs are observed in ≤ 1 patient out of 6 patients treated, this cohort will be expanded as described below. If a DLT is observed in one of the first 3 patients, after analysis of the cytokine data, 3 additional patients can be accrued to this cohort. If more than 1 out of 6 patients in this cohort experience a DLT, this cohort will be closed. Subsequent dose reductions will be discussed with the FDA prior to initiation.

If ≤ 1 patient in 6 patients treated develops a DLT then the cohort will be expanded to a total of 18 evaluable patients, subject to not exceeding 3 patients in total with a DLT.

Starting with Amendment S

The primary endpoints of this trial are to determine the safety and feasibility of the administration of cryopreserved anti-CD19-CAR engineered peripheral blood lymphocytes following a non-myeloablative conditioning regimen in patients with B-cell malignancies. The secondary endpoints are to determine the *in vivo* survival of the cryopreserved anti-CD19-CAR-transduced T-cells and determine if the treatment regimen cause regression of B-cell malignancies.

Starting with Amendment S, Cohort 10 was closed and only two dose cohorts will be open, including a de-escalation cohort: cohorts 11, 11D, and 12.

Initially 3 patients will be enrolled in Cohort 11. If 1 patient experiences a DLT the cohort will be expanded to 6 patients, if no additional DLTs are seen, patients will enroll in Cohort 12. If less than or equal to one DLT is seen in the first 6 patients enrolled in Cohort 12, the cohort will be expanded to a total of 12 patients as long as less than 1/3 of patients have a DLT in this cohort in order to further characterize the safety of the maximum tolerated dose.

If 2 or more DLTs are encountered in Cohort 12, Cohort 11 will be expanded to a maximum of 12 patients provided that less than 1/3 of patients have a DLT in this cohort.

If more than one in six patients experiences a DLT in Cohort 11, patients will be enrolled in Cohort 11D. If less than or equal to one DLT is seen in the first 6 patients enrolled in Cohort 11D, the cohort will be expanded to a total of 12 patients as long as less than 1/3 of patients have a DLT in this cohort in order to further characterize the safety of the maximum tolerated dose.

Cohort	Treatment Regimen		
	<i>NMA Chemotherapy Regimen (as specified in protocol)</i>	<i>Cell Dose: Determined Prior to Cryopreservation</i>	<i>IL-2 Dose</i>
11	Yes	2.0x10 ⁶ CAR positive cells/kg (± 20%)	None
11D (De-escalation Cohort)	Yes	1.0x10 ⁶ CAR positive cells/kg (± 20%)	None
12	Yes	6.0x10 ⁶ CAR positive cells/kg (± 20%)	None

With the approval of Amendment X, Cohort 13 was completed and Cohort 14 has been opened.

With the approval of Amendment Y, the dose de-escalation for Cohort 14 was corrected to state that Cohort 11 would be the de-escalation cohort. With the approval of Amendment Z, the Dose Escalation and DLT sections have been revised.

The primary endpoints of this trial are to determine the safety and feasibility of the administration of cryopreserved anti-CD19-CAR engineered peripheral blood lymphocytes following a non-myeloablative conditioning regimen in patients with B-cell malignancies. The secondary endpoints are to determine the *in vivo* survival of the cryopreserved anti-CD19-CAR-transduced T-cells and determine if the treatment regimen cause regression of B-cell malignancies.

Refer to Section [3.1.1](#) for additional details.

The maximum tolerated cell dose is the highest dose at which ≤ 1 of 6 patients experienced a DLT or the highest dose level studied if DLTs are not observed at any of the dose levels.

At the conclusion of the study, toxicities will be tabulated according to worst grade per patient of each type of toxicity, and the fraction experiencing a DLT will be reported, along with appropriate confidence intervals.

In order to complete enrollment to this protocol, up to 72 patients may be needed. This includes 37 patients already accrued to this study, up to 14 additional patients for Cohort 11 (four patients are already enrolled in this cohort), and either up to 18 patients for Cohort 11D, or, an additional 16 patients for Cohort 14, and 3 additional patients in the event patients need to be replaced for any reason. Thus, at an accrual rate of 6-12 patients per year, up to 3 additional years may be required to enroll up to 72 patients onto this trial.

9 COLLABORATIVE AGREEMENTS

We have established a Cooperative Research and Development Agreement (CRADAs #02716 and #03168) with Kite Pharma, Inc., and will be sharing data with them.

10 HUMAN SUBJECTS PROTECTIONS

10.1 RATIONALE FOR SUBJECT SELECTION

The patients to be entered in this protocol have B-cell malignancies which are refractory to standard therapy, and limited life expectancies. Subjects from both genders and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one group compared to another. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on the one hand and the need to explore gender and ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to gender or to ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.

10.2 PARTICIPATION OF CHILDREN

The use of the non-myeloablative regimen in this protocol is a major procedure which entails serious discomforts and hazards for the patient, such that fatal complications are possible. It is therefore only appropriate to carry out this experimental procedure in the context of life threatening metastatic cancer. Since the efficacy of this experimental procedure is unknown, it does not seem reasonable to expose children to this risk without further evidence of benefit. Should results of this study indicate efficacy in treating B-cell malignancies, which is not responsive to other standard forms of therapy, future research can be conducted in the pediatric population to evaluate potential benefit in that patient population.

10.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (Section 10.5), all subjects \geq age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the “NIH Advance Directive for Health Care and Medical Research Participation” form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MAS Policy 87-4 and OHSRP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

10.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

The experimental treatment has a chance to provide clinical benefit though this is unknown. The risks in this treatment are detailed in Section 1.2.9. The goal of this study is to improve upon the number of patients who may benefit from adoptive cell therapy by using patients' own transduced T-cells without the need to identify anti-tumor T-cells uniquely from each patient as was required in several prior protocols. The success of this effort cannot be predicted at this time. Because all patients in this protocol have B-cell malignancies and limited life expectancies the potential benefit is thought to outweigh the potential risks.

10.5 RISK/BENEFIT ANALYSIS

Because all patients in this protocol have B-cell malignancies and limited life expectancies, the potential benefit is thought to outweigh the potential risks. The risk/benefit analysis for adults with the capacity to consent, as well as for adults who may become unable to provide consent, is greater than minimal risk with the prospect of direct benefit.

10.6 CONSENT PROCESS AND DOCUMENTATION

Patients initially sign a consent document when they agree to have PBMC obtained for study and growth on protocol 03-C-0277. If the lymphocytes can be generated for infusion and the patient meets the thorough screening for eligibility, the patient, with family members or friends at the request of the patient, will be presented with a detailed description of the protocol treatment. The specific requirements, objectives, and potential advantages and disadvantages will be presented. The informed consent document is given to the patient, who is requested to review it and to ask questions prior to agreeing to participate in the treatment portion of this protocol. The patient is reassured that participation on trial is entirely voluntary and that he/she can withdraw or decide against treatment at any time without adverse consequences. The Principal Investigator, associate investigator, or clinical fellow is responsible for obtaining written consent from the patient.

10.6.1 Telephone Consent

The informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witness to the subject's signature will sign and date the consent.

The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone.

A fully executed copy will be returned via mail for the subject's records.

The informed consent process will be documented on a progress note by the consenting investigator and a copy of the informed consent document and note will be kept in the subject's research record.

11 PHARMACEUTICAL INFORMATION

11.1 INVESTIGATIONAL REGIMEN

11.1.1 Anti-CD19 CAR transduced PBL

The procedure for expanding the human PBL and the Certificate of Analysis are similar to those approved by the Food and Drug Administration, and used at the NCI in ongoing protocols

evaluating cell therapy in the Surgery Branch (most recently 07-C-0174, 07-C-0175, 08-C-0121, 08-C-0155, and 08-C-0162), and is included in **Appendix 1** and in the IND submission for these cells. The PBL will be transduced with retroviral supernatant containing the anti-CD19 CAR.

11.1.1.1 Retroviral Vector Containing the anti-CD19 CAR Gene

The retroviral vector supernatant (PG13-CD19-H3) encoding a chimeric antigen receptor (CAR) directed against the B-cell antigen, CD19, was prepared and preserved following cGMP conditions in the Surgery Branch Vector Production Facility (SBVPF). The retroviral vector utilizes the MSGV1 retroviral vector backbone and consists of 7026 bps including the 5' LTR from the murine stem cell virus (promoter), packaging signal including the splicing donor (SD) and splicing acceptor sites, FMC63-based (anti-CD19 FMC63-28) CAR protein containing a signal peptide (human GM-CSF receptor), FMC63 light chain variable region (FMC63 VL), linker peptide, FMC63 heavy chain variable region (FMC63 VH), CD28 (hinge, transmembrane and cytoplasmic region), and TCR zeta (cytoplasmic region), followed by the murine stem cell virus 3'LTR. The physical titer will be determined by RNA dot blot according to sponsor certificate.

The supernate will be stored at SBVPF upon the completion of production at -80°C or shipped on dry ice and stored at Cryonix, Rockville, MD. Both storage facilities are equipped with around-the-clock temperature monitoring. Upon request, supernatant will be delivered on dry ice to be used in *in vitro* transductions of PBL. There will be no re-use of the same unit of supernate for different patients. Retroviral titer has been shown to be stable after immediate thawing and immediate administration (coating the tissue culture wells previously coated with Retronectin). Handling of the vector should follow the guidelines of Biosafety Level-2 (BSL-2). The specific guidelines for Biosafety Level-2 (BSL-2) can be viewed at <http://bmbi.od.nih.gov/sect3bsl2.htm>.

11.1.1.2 OKT3

OKT3 will be obtained by the Surgery Branch Laboratory from commercial sources.

Formulation: Muromonab-CD3 (Ortho), NSC #618843, is provided as a sterile, clear, colorless solution at a concentration of 1 mg/mL in 5 mL ampoules. The solution may contain a few fine, translucent protein particles. The antibody is dissolved in a buffered solution at pH of 6.5 to 7.5. The solution contains 2.25 mg of monobasic sodium phosphate, 9 mg of dibasic sodium phosphate, 43 mg of sodium chloride and 1 mg of polysorbate 80 per 5 mL of water for injection.

Storage/Stability: Ampules should be stored in a refrigerator at $2-8^{\circ}\text{C}$. Solution should not be frozen or shaken. Each ampule bears an expiration date.

11.1.2 Fludarabine

(Please refer to package insert for complete product information)

Description: Fludarabine phosphate is a synthetic purine nucleoside that differs from physiologic nucleosides in that the sugar moiety is arabinose instead of ribose or deoxyribose. Fludarabine is a purine antagonist antimetabolite.

How Supplied: It will be purchased by the NIH Clinical Pharmacy Department from commercial sources. Fludarabine is supplied in a 50 mg vial as a fludarabine phosphate powder in the form of a white, lyophilized solid cake.

Stability: Following reconstitution with 2 mL of sterile water for injection to a concentration of 25 mg/mL, the solution has a pH of 7.7. The fludarabine powder is stable for at least 18 months at 2-8°C; when reconstituted, fludarabine is stable for at least 16 days at room temperature. Because no preservative is present, reconstituted fludarabine will typically be administered within 8 hours. Specialized references should be consulted for specific compatibility information. Fludarabine is dephosphorylated in serum, transported intracellularly and converted to the nucleotide fludarabine triphosphate; this 2-fluoro-ara-ATP molecule is thought to be required for the drug's cytotoxic effects. Fludarabine inhibits DNA polymerase, ribonucleotide reductase, DNA primase, and may interfere with chain elongation, and RNA and protein synthesis.

Storage: Intact vials should be stored refrigerated (2-8°C).

Administration: Fludarabine is administered as an IV infusion in 100 mL 0.9% sodium chloride, USP over 15 to 30 minutes. The doses will be based on body surface area (BSA). If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in [Appendix 4](#).

Toxicities: At doses of 25 mg/m²/day for 5 days, the primary side effect is myelosuppression; however, thrombocytopenia is responsible for most cases of severe and life-threatening hematologic toxicity. Serious opportunistic infections have occurred in CLL patients treated with fludarabine. Hemolytic anemia has been reported after one or more courses of fludarabine with or without a prior history of a positive Coomb's test; fatal hemolytic anemia has been reported. In addition, bone marrow fibrosis has been observed after fludarabine therapy. Other common adverse effects include malaise, fever, chills, fatigue, anorexia, nausea and vomiting, and weakness. Irreversible and potentially fatal central nervous system toxicity in the form of progressive encephalopathy, blindness, and coma is only rarely observed at the currently administered doses of fludarabine. More common neurologic side effects at the current doses of fludarabine include weakness, pain, malaise, fatigue, paresthesia, visual or hearing disturbances, and sleep disorders. Adverse respiratory effects of fludarabine include cough, dyspnea, allergic or idiopathic interstitial pneumonitis. Tumor lysis syndrome has been rarely observed in fludarabine treatment of CLL. Treatment on previous adoptive cell therapy protocols in the Surgery Branch have caused persistently low (below 200) CD4 counts, and one patient developed polyneuropathy manifested by vision blindness, and motor and sensory defects.

11.1.3 Cyclophosphamide

(Refer to FDA-approved package insert for complete product information)

Description: Cyclophosphamide is a nitrogen mustard-derivative alkylating agent. Following conversion to active metabolites in the liver, cyclophosphamide functions as an alkylating agent; the drug also possesses potent immunosuppressive activity. The serum half-life after IV administration ranges from 3-12 hours; the drug and/or its metabolites can be detected in the serum for up to 72 hours after administration.

How Supplied: Cyclophosphamide will be obtained from commercially available sources by the Clinical Center Pharmacy Department.

Stability: Following reconstitution as directed with sterile water for injection, cyclophosphamide is stable for 24 hours at room temperature or 6 days when kept at 2-8°C.

Administration: It will be diluted in 250 mL D5W and infused over one hour. The dose will be based on the patient's body weight. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in [Appendix 4](#).

Toxicities: Hematologic toxicity occurring with cyclophosphamide usually includes leukopenia and thrombocytopenia. Anorexia, nausea and vomiting, rash and alopecia occur, especially after high-dose cyclophosphamide; diarrhea, hemorrhagic colitis, infertility, and mucosal and oral ulceration have been reported. Sterile hemorrhagic cystitis occurs in about 20% of patients; severity can range from microscopic hematuria to extensive cystitis with bladder fibrosis. Although the incidence of hemorrhagic cystitis associated with cyclophosphamide appears to be lower than that associated with ifosfamide, mesna (sodium 2-mercaptoethanesulfonate) has been used prophylactically as a uroprotective agent in patients receiving cyclophosphamide. Prophylactic mesna is not effective in preventing hemorrhagic cystitis in all patients. Patients who receive high dose cyclophosphamide may develop interstitial pulmonary fibrosis, which can be fatal. Hyperuricemia due to rapid cellular destruction may occur, particularly in patients with hematologic malignancy. Hyperuricemia may be minimized by adequate hydration, alkalinization of the urine, and/or administration of allopurinol. If allopurinol is administered, patients should be watched closely for cyclophosphamide toxicity (due to allopurinol induction of hepatic microsomal enzymes). At high doses, cyclophosphamide can result in a syndrome of inappropriate antidiuretic hormone secretion; hyponatremia with progressive weight gain without edema occurs. At high doses, cyclophosphamide can result in cardiotoxicity. Deaths have occurred from diffuse hemorrhagic myocardial necrosis and from a syndrome of acute myopericarditis; in such cases, congestive heart failure may occur within a few days of the first dose. Other consequences of cyclophosphamide cardiotoxicity include arrhythmias, potentially irreversible cardiomyopathy, and pericarditis. Other reported adverse effects of cyclophosphamide include headache, dizziness, and myxedema; faintness, facial flushing, and diaphoresis have occurred following IV administration. Mesna (sodium 2-mercaptoethanesulfonate; given by IV injection) is a synthetic sulfhydryl compound that can chemically interact with urotoxic metabolites of cyclophosphamide (acrolein and 4-hydroxycyclophosphamide) to decrease the incidence and severity of hemorrhagic cystitis.

11.2 SUPPORT MEDICATIONS

11.2.1 Mesna (Sodium 2-mercaptoethanesulfonate, Mesnum, Mesnex, NSC-113891)

(Please refer to the FDA-approved package insert for complete product information)

Description: Mesna will be obtained commercially by the Clinical Center Pharmacy Department and is supplied as a 100 mg/mL solution.

Storage: Intact ampoules are stored at room temperature.

Stability: Diluted solutions (1-20 mg/mL) are physically and chemically stable for at least 24 hours under refrigeration. Mesna is chemically stable at room temperature for 48-72 hours in D5W, 48-72 hour in D5W/0.45% NaCl, or 24 hours in 0.9% NaCl.

Administration: Dilute to concentrations less than or equal to 20 mg mesna/mL fluid in D5W or 0.9% NaCl and to be administered intravenously as a continuous infusion. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in [Appendix 4](#).

Toxicities: Nausea, vomiting, and diarrhea.

11.2.2 Filgrastim (Granulocyte Colony-Stimulating Factor, G-CSF, Filgrastim, Neupogen)

Filgrastim will be obtained commercially by the Clinical Center Pharmacy Department and is supplied in 300 ug/mL and 480 ug/1.6 mL vials. G-CSF should be refrigerated and not allowed to freeze. The product bears the expiration date. The product should not be shaken. It is generally stable for at least 10 months when refrigerated. The appropriate dose is drawn up into a syringe. G-CSF will be given as a daily subcutaneous injection. The side effects of G-CSF are skin rash, myalgia and bone pain, an increase of preexisting inflammatory conditions, enlarged spleen with occasional associated low platelet counts, alopecia (with prolonged use) elevated blood chemistry levels.

11.2.3 Trimethoprim and Sulfamethoxazole Double Strength (TMP / SMX DS)

TMP/SMX DS will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used for the prevention of PCP pneumonia. The oral dose is 1 tablet PO daily three times a week (MUST be on non-consecutive days) beginning on day -7 and continuing for at least 6 months and until the CD4 count is greater than 200 on 2 consecutive lab studies. Like other sulfa drugs, TMP/SMX DS can cause allergies, fever, photosensitivity, nausea, and vomiting. Allergies typically develop as a widespread itchy red rash with fever eight to fourteen days after beginning the standard dose. Neutropenia, a reduction in the number of neutrophils, can also occur.

11.2.3.1 Aerosolized Pentamidine in Place of TMP/SMX DS

Patients with sulfa allergies will receive aerosolized Pentamidine 300 mg per nebulizer with one week prior to admission and continued monthly until the CD4 count is above 200 on two consecutive follow up lab studies and for at least 6 months post chemotherapy. Pentamidine Isethionate will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prevent the occurrence of PCP infections. It is supplied in 300 mg vials of lyophilized powder and will be administered via nebulizer. Toxicities reported with the use of Pentamidine include metallic taste, coughing, bronchospasm in heavy smokers and asthmatics; increased incidence of spontaneous pneumothorax in patients with previous PCP infection or pneumatoceles, or hypoglycemia.

11.2.4 Herpes Virus Prophylaxis

11.2.4.1 Valacyclovir (Valtrex)

Valacyclovir will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used orally to prevent the occurrence of herpes virus infections in patients with positive HSV serology. It is supplied in 500 mg tablets. Valacyclovir will be started the day after the last dose of fludarabine at a dose of 500 mg orally daily if the patient is able to tolerate oral intake. See package insert for dosing adjustments in patients with renal impairment. Common side effects include headache, upset stomach, nausea, vomiting, diarrhea or constipation. Rare serious side effects include hemolytic uremic syndrome and thrombotic thrombocytopenic purpura.

11.2.4.2 Acyclovir

Acyclovir will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prevent the occurrence of herpes virus infections in patients who cannot take oral medications. It is supplied as powder for injection in 500 mg/vials. Reconstitute

in 10 mL of sterile water for injection to a concentration of 50 mg/mL. Reconstituted solutions should be used within 12 hours. IV solutions should be diluted to a concentration of 7mg/mL or less and infused over 1 hour to avoid renal damage. Reversible renal insufficiency has been reported with IV but not oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs have been reported with higher doses of acyclovir. Should this occur, a dosage adjustment will be made or the drug will be discontinued. Stomach upset, headache or nausea, rash or hives; peripheral edema; pain, elevated liver function tests; and leukopenia, diarrhea, lymphadenopathy, myalgias, visual abnormalities and elevated creatinine have been reported. Hair loss from prolonged use has been reported. Acyclovir will not be used concomitantly with other nucleoside analogs which interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling.

11.2.5 Fluconazole

Fluconazole will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prophylax against fungal infections. It is available in 200 mg tablets. It can cause headache, nausea, vomiting, diarrhea or abdominal pain, and liver damage which may be irreversible. It can cause rashes and itching, which in rare cases has caused Stevens Johnson Syndrome. It has several significant drug interactions. The package insert should be consulted prior to prescribing. For IV administration in patients who cannot tolerate the oral preparation, Fluconazole comes in 2 mg/mL solution for injection, and prepared according to Clinical Center Pharmacy standard procedures. It should be administered at a maximum IV rate of 200 mg/hr.

11.2.6 Ondansetron Hydrochloride

Ondansetron hydrochloride will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to control nausea and vomiting during the chemotherapy preparative regimen. It can cause headache, dizziness, myalgias, drowsiness, malaise, and weakness. Less common side effects include chest pain, hypotension, pruritis, constipation and urinary retention. Consult the package insert for specific dosing instructions.

11.2.7 Allopurinol

Allopurinol will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used as prophylaxis or treatment of patients with or at high risk for Tumor Lysis Syndrome. Dosage is approximately 100 mg/m²/dose po TID (maximum dose 200 mg TID). The most common side effects include hypersensitivity, rash, nausea, vomiting, renal insufficiency, and hepatic dysfunction. Allopurinol should be stopped immediately if rash develops. Consult the package insert for a complete list of all side effects.

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13 TABLES, FIGURES, AND APPENDICES**13.1 TABLE 1: SURGERY BRANCH CELL THERAPY STUDIES**

Data as of 09/30/2008

Study #/ Disease	Cellular Product Administered	Arms (n)	Chemotherapy, Cytokines, and Immunizations	# of Cells	Response	Reference
98-C-0095 in patients with metastatic melanoma	Cloned Peripheral Blood Lymphocytes (PBL)/ Tumor infiltrating lymphocytes (TIL)	1. Cloned PBL/TIL intravenously (12)	None	1.5 to 34.8x10 ⁹ cells	NR ^d	Dudley, ME, <i>et al.</i> 2001
		2. IV Cloned PBL/TIL intravenously (6 ^a)	SQ IL-2 (125,000 IU/kg/d X 12 d)			
		3. IV Cloned PBL/TIL intravenously (6 ^b)	HD IL-2 (720,000 IU/kg 3X/d to tolerance (max 12)			
		4. IV Cloned PBL/TIL (3 ^c)	Gp100:209-217(210M)			
99-C-0158 in patients with metastatic melanoma	Tumor infiltrating lymphocytes (TIL)	1. <i>In vitro</i> expanded cloned T-cells intravenously (3)	30 mg/kg cyclophosphamide X2 days, 25 mg/kg fludarabine X5 days	0.9x10 ⁹ to 24.2x10 ⁹ cells (avg. 10.4x10 ⁹)	NR	Dudley, ME, <i>et al.</i> 2002
		2. <i>In vitro</i> expanded cloned T-cells intravenously (3)	60 mg/kg cyclophosphamide X2 days, 25 mg/kg fludarabine X5 days		NR	
		3. <i>In vitro</i> expanded cloned T-cells intravenously (3)	60 mg/kg cyclophosphamide X2 days, 25 mg/kg fludarabine X5 days plus IV IL-2 (72,000 IU/kg 3X/day X5 days)	0.9x10 ⁹ to 24.2x10 ⁹ cells (avg. 10.4x10 ⁹)	NR	
		4. <i>In vitro</i> expanded cloned T-cells intravenously (6)	[60 mg/kg cyclophosphamide X2 days, 25 mg/kg fludarabine X5 days] ^g plus IV IL-2 (720,000 IU/kg 3X/day to tolerance (max 12)		NR	
		5. Autologous tumor reactive REP ^d e bulk TIL cells intravenously (35)	Chemotherapy ^g plus IV HD IL-2 [(720,000 IU/kg 3X/day to tolerance (max 15)] ^h with or without immunization with gp100:209-217(210M) or MART-1:26-35(27L) in Montanide ISA-51 TM QD X5, then Qwk X3.	1.1 to 16.0x10 ¹⁰ cells (avg. 6.3x10 ¹⁰)	18/35 (51%) ^f 3 CRs 15 PRs	Dudley, ME, <i>et al.</i> 2003, Dudley, ME, <i>et al.</i> 2005
		6. Autologous tumor reactive REP ^d e bulk TIL cells intravenously (6)	Chemotherapy ^g plus Low Dose IL-2 ^k with or without immunization with gp100:209-217(210M) peptide or MART-1:26-	8.3x10 ⁹ to 52.9x10 ⁹ cells (avg. 33.7x10 ⁹)	3PR 2NR 1 TE ^j	

			35(27L) peptide in Montanide ISA-51™ QD X5, then Qwk X3.			
04-C-0181 in patients with metastatic melanoma	Gp100 TCR engineered T-cells (PBL or TIL)	1. Escalating doses of anti-gp100 TCR transduced PBL (3)	Chemotherapy ^g plus IV HD IL-2 ^h with 1mg gp100:209-217(210M) in Montanide ISA-51™ QD X5, then Qwk X3	Up to 30x10 ⁹	NR	NA
		2. Anti-gp100 TCR CD8+ enriched PBL (8)	Chemotherapy ^g plus IV HD IL-2 ^h with 6X10 ⁹ pfu rFgp100P209 IV; rFgp100P209 IV and IL-2 repeated 28 days later.	Up to 30x10 ⁹	NR	
		3. Escalating doses of anti-gp100 TCR transduced TIL (3)	Chemotherapy ^g plus IV HD IL-2 ^h with 6X10 ⁹ pfu rFgp100P209 IV; rFgp100P209 IV and IL-2 repeated 28 days later.	Up to 30x10 ⁹	1 PR ⁱ	
04-C-0251 in patients with metastatic melanoma	MART-1 TCR engineered T-cells (PBL or TIL)	1. Escalating doses of anti-MART-1 TCR transduced PBL (18)	Chemotherapy ^g plus IV HD IL-2 ^h with MART-1:26-35(27L) in Montanide ISA-51™ QD X5, then Qwk X3.	Up to 30x10 ⁹	2PRs ⁱ	Morgan, <i>et al.</i> Science, 2006 Oct 6;314(5796): 126-9
		2. Escalating doses of anti-MART-1 CD8+ TCR transduced PBL (6)	Chemotherapy ^g plus IV HD IL-2 ^h with MART-1:27-35 in Montanide ISA-51™ QD X5, then Qwk X3	Up to 30x10 ⁹	1 PR ⁱ	
		3. Escalating doses of anti-MART-1 CD8+ TCR transduced PBL (8)	Chemotherapy ^g plus IV HD IL-2 ^h with MART-1:26-35(27L) in Montanide ISA-51™ QD X5, then Qwk X3	Up to 30x10 ⁹	1 PR ⁱ	
		4. Escalating doses of anti-MART-1 TCR transduced TIL (3)	Chemotherapy ^g plus IV HD IL-2 ^h with MART-1:26-35(27L) in Montanide ISA-51™ QD X5, then Qwk X3	Up to 30x10 ⁹	NR	
		5. Escalating doses of anti-MART-1 TCR transduced PBL plus 1200 TBI (4)	Chemotherapy ^g and 1200 TBI plus IV HD IL-2 ^h with MART-1:26-35(27L) in Montanide ISA-51™ QD X5, then Qwk X3	Up to 30x10 ⁹	NR	
07-C-0003 in melanoma/ renal cell or other histologies	Anti-p53 TCR engineered T-cells (PBL)	1. Melanoma or renal cell cancer: Up to 50 X 10 ⁹ cells (PBL) (2)	Chemotherapy ^g plus IV HD IL-2 ^h	Up to 50x10 ⁹	NR	NA
		2. Other histologies: Up to 50 X 10 ⁹ cells (PBL) (10)	Chemotherapy ^g plus IV HD IL-2 ^h	Up to 50x10 ⁹	1 PR ⁱ	

07-C-0174 in patients with metastatic melanoma	Anti- gp100(154) TCR engineered T- cells (PBL)	Anti-gp100(154) TCR transduced PBL at a dose ranging from 0.5 X 10 ⁹ cells up to 300 x10 ⁹ (19)	Chemotherapy ^g plus IV HD IL-2 ^h	1.7x10 ⁹ to 110.0x10 ⁹ cells (avg. 31.5x10 ⁹)	3 PR 14 NR 2 TE ^j	NA
07-C-0175 in patients with metastatic melanoma	Anti-MART-1 F5 TCR engineered T- cells (PBL or TIL)	Anti-MART-1 F5 TCR transduced PBL at a dose ranging from 0.5 X 10 ⁹ cells up to 300 x10 ⁹ cells (21)	Chemotherapy ^g plus IV HD IL-2 ^h	1.45x10 ⁹ to 110.0x10 ⁹ cells (avg. 34.2x10 ⁹)	6 PR 12 NR 3 TE ^j	NA
		Anti-MART-1 F5 TCR transduced TIL at a dose ranging from 0.5 X 10 ⁹ cells up to 300 x10 ⁹ cells	Chemotherapy ^g plus IV HD IL-2 ^h	84x10 ⁹ cells	1 TE	NA
07-C-0176 in patients with metastatic melanoma	Young TIL	Young TIL at a dose ranging from 1.0 X 10 ⁹ cells up to 300 x10 ⁹ cells (26)	Chemotherapy ^g plus IV HD IL-2 ^h	11.1x10 ⁹ to 124x10 ⁹ cells (avg. 55.6x10 ⁹)	3 PR 19 NR 2 TE ^j 2 NT ^j	NA

^a Previously treated with cells alone

^b Five previously treated with cells alone, one new patient for first cell treatment

^c All three patients had previously been treated with cell alone and cells with IL-2

^d NR: No Response

^e Rapid Expansion Protocol

^f Responses defined: CR (complete response) is disappearance of all clinical evidence of disease; PR (partial response) defined as > 50% reduction in the sum of the products of the perpendicular diameters for at least one month and no increase in any lesion and no new lesions.

^g Chemotherapy at maximum dose: 60 mg/kg cyclophosphamide X2 days, 25 mg/kg fludarabine X5 days

^h High dose (HD) IL-2: 720,000 IU/kg 3X/day to tolerance (max 15)

ⁱ Responses defined according to RECIST criteria

^j TE: Too early to evaluate, NT: not treated

^k Low dose IL-2: 250,000 IU/kg subcutaneously daily for 5 days. After a two day rest, IL-2 will be administered at a dose of 125,000 IU/kg subcutaneously daily for 5 days for the next five weeks (2 days rest per week).

13.2 TABLE 2: TIME IN HOSPITAL AND NON-HEMATOLOGICAL GRADE 3 AND 4 TOXICITIES RELATED TO LYMPHODEPLETING CHEMOTHERAPY AND CELL TRANSFER

Attribute measured	Duration, Number or Type	Number of Patients (%)
Days in Hospital ¹	6-10	6 (17%)
	11-15	18 (51%)
	16-20	4 (11%)
	21-25	7 (20%)
pRBC Transfusions	0	2 (6%)
	1-5	18 (51%)
	6-10	13 (37%)
	11-15	2(6%)
Platelet Transfusions	0	6 (17%)
	1-5	21 (60%)
	6-10	5 (14%)
	11-15	2 (6%)
	16-20	1 (3%)
Autoimmunity	Uveitis	5 (14%)
	Vitiligo	13 (37%)
Opportunistic Infections	Herpes zoster	3 (9%)
	Pneumocystis pneumonia	2 (6%)
	EBV-B cell lymphoma	1 (3%)
	RSV pneumonia	1 (3%)
Other	Febrile neutropenia	13 (37%)
	Intubated for dyspnea	3 (9%)
	Cortical blindness	1 (3%)

¹Measured from the day of cell administration to discharge

13.3 FIGURE 1: CANCER REGRESSION IN TWO PATIENTS

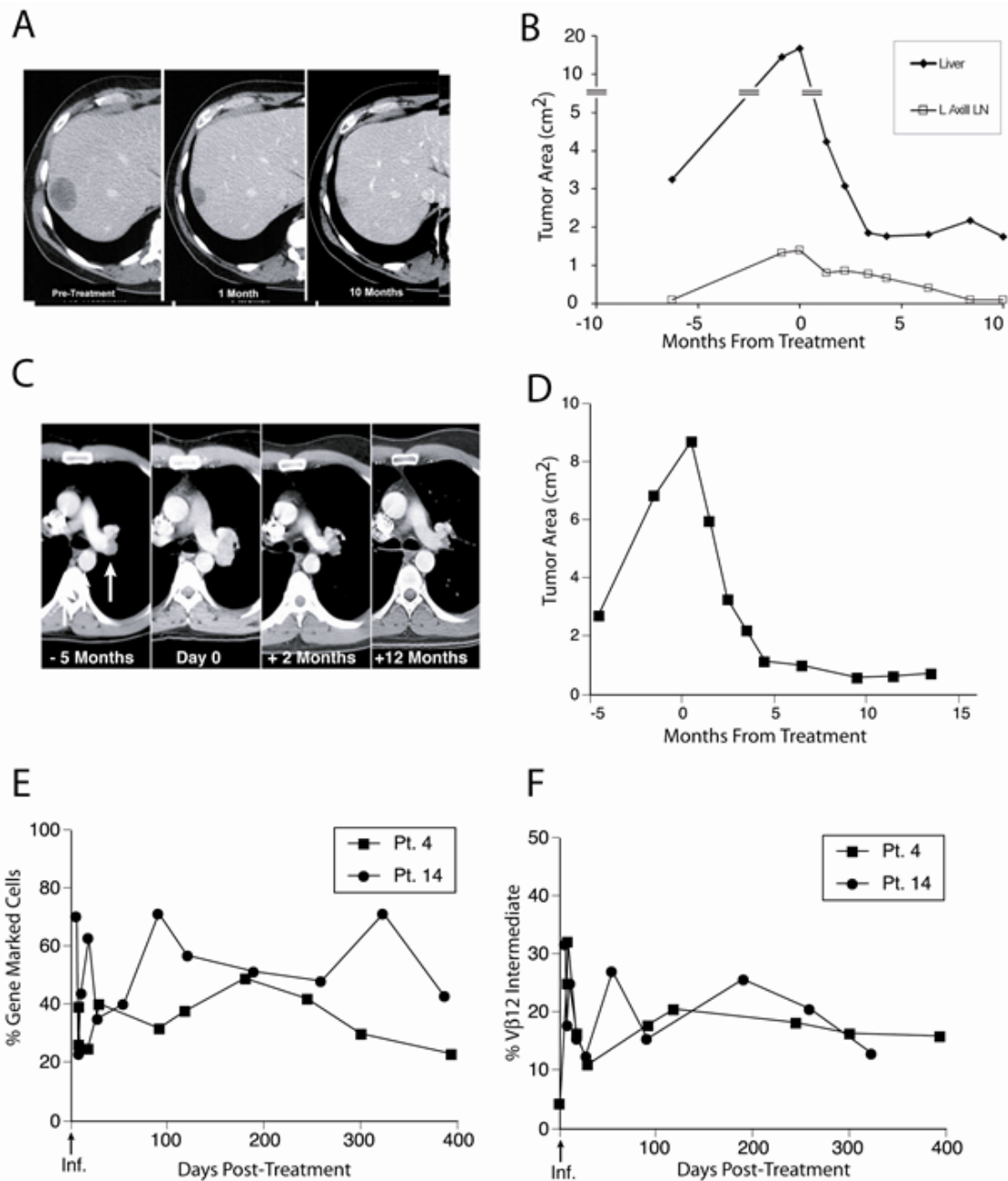


Figure 1. Cancer regression in two patients. **A.** CT images of patient 4 liver metastasis; pre-treatment, one month and 10 months post-treatment with TCR engineered T-cells. **B.** Size of liver and axillary tumors and tempo of regression of tumor sites in patient 4 (treatment time = day 0). **C.** CT images of patient 14 hilar lymph node metastasis; pre-treatment, beginning of treatment (Day 0), and two months and 12 months post-treatment. **D.** Size of tumor and tempo of regression in patient 14. **E.** Quantitation of gene marked cells in patients 4 and 14 PBMC was determined by real-time quantitative PCR. Day of infusion (Inf.) indicated by arrow. **F.** The percentage of CD8⁺/Vβ12 cells in the intermediate gate in the circulation of patients 4 and 14.

13.4 FIGURE 1: NORMAL PBMC CAN BE TRANSDUCED WITH AN ANTI-CD19 CAR

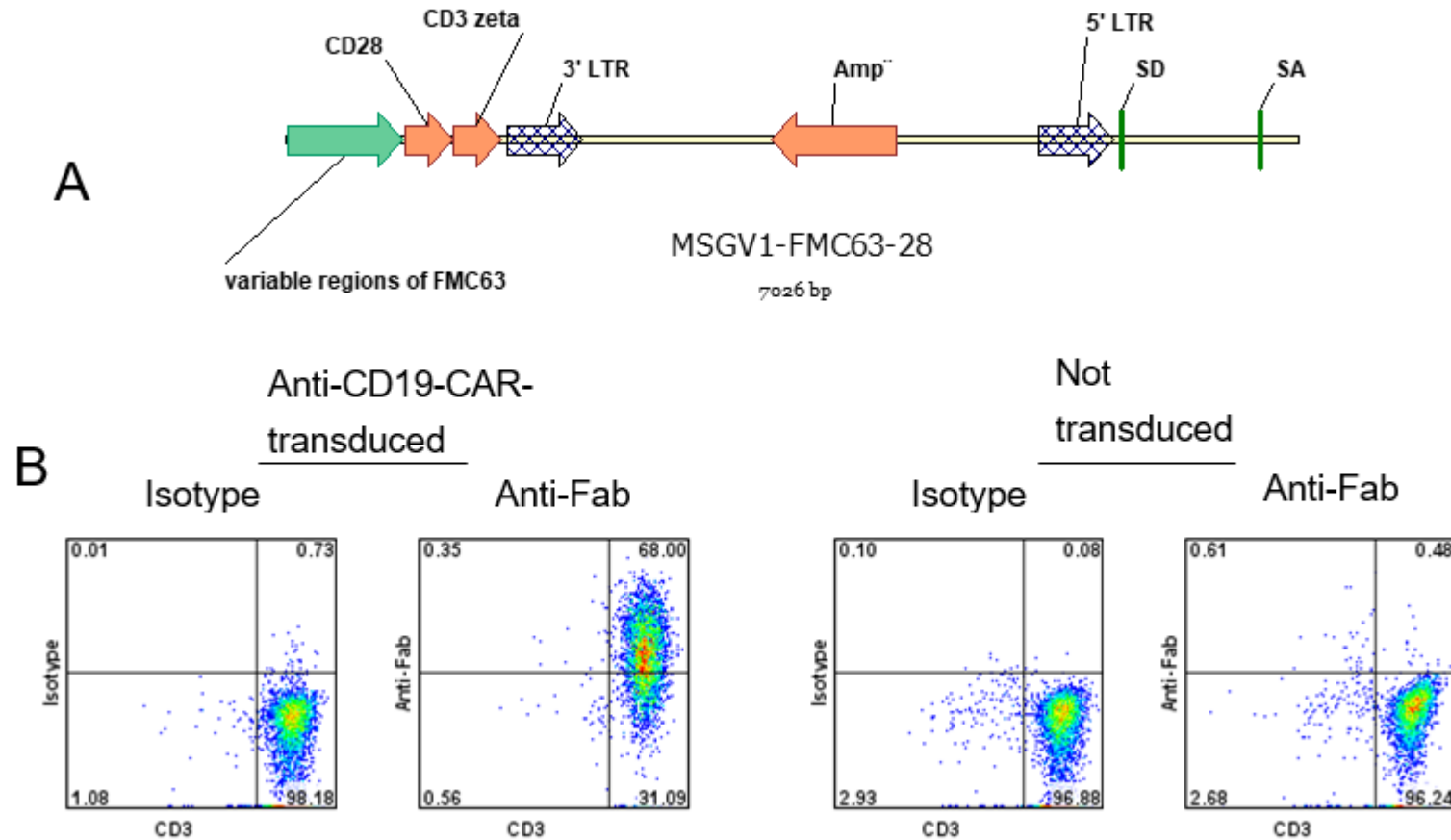


Figure 2. Normal PBMC can be transduced with an anti-CD19 CAR. **A.** Linearized plasmid map of the MSGV1-FMC63-28 gamma-retroviral vector that encodes the FMC63-28 anti-CD19 chimeric antigen receptor. **B.** Expression of the anti-CD19 chimeric receptor can be detected on the surface of transduced T-cells by staining with a goat-anti-mouse Fab antibody. Plots are gated on live lymphocytes.

13.5 FIGURE 2: PBMC TRANSDUCED WITH ANTI-CD19 CAR RECOGNIZE CD19-EXPRESSING TARGET CELLS

Effector cells	CD19+ targets			CD19 negative targets					Effectors alone
	SupB15	NALM6	Toledo	624	A549	TC71	CEM	K562	
Anti-CD19 CAR	14800	15150	15150	38	19	63	8	24	4
SP6 Control CAR	23	35	36	83	124	46	22	184	14
Donor 1 Nontransduced	13	54	39	7	5	7	16	8	3

Figure 3. PBMC that were transduced with an anti-CD19 CAR specifically recognize CD19-expressing target cells. 100,000 effector cells were cultured overnight with 100,000 target cells, and an interferon- γ ELISA was performed. All values are pg/mL of interferon- γ (mean of duplicate wells). Effector cells were either anti-CD19-CAR-transduced T-cells, T-cells transduced with the control CAR SP6, or non-transduced T-cells from the same patient stimulated with OKT3 in the same manner. SupB15 and NALM6 are CD19-expressing leukemia cell lines, Toledo is a CD19-expressing large cell lymphoma cell line. 624, A549, TC71, CEM and K562 are immortalized CD19 negative cell lines. The control CAR SP6 is specific for the chemical 2,4,6-TNP.

13.6 FIGURE 3: T-CELLS FROM A CLL PATIENT POST FLUDARABINE/RITUXIMAB THERAPY**A**

Effector cells	CD19+ targets		CD19 negative targets			Effectors alone
	CLL1	CLL2	Allogeneic T cell	CEM	K562	
anti-CD19 CAR-transduced	72,000	47,000	1945	32	490	24
Nontransduced	132	345	305	36	52	18

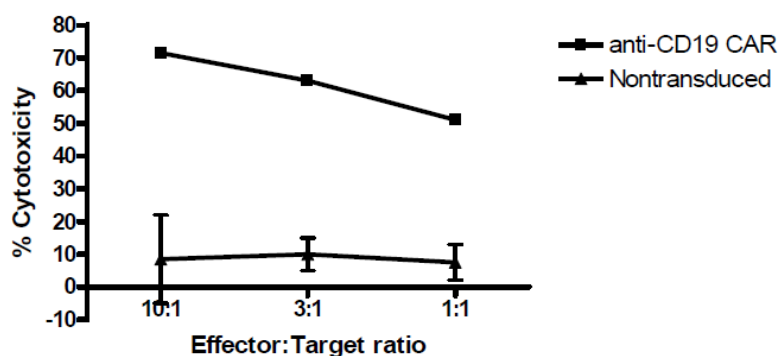
B

Figure 4. T-cells from a CLL patient post fludarabine/rituximab therapy can be transduced with an anti-CD19 CAR and then produce interferon- γ in response to a primary allogeneic CLL cells and kill primary allogeneic CLL cells. A. 100,000 effector cells were cultured overnight with 100,000 target cells, and an interferon- γ ELISA was performed. All values are pg/mL of interferon- γ (mean of duplicate wells). Effector cells were either anti-CD19-CAR-transduced T-cells from a CLL patient or non-transduced T-cells from the same patient stimulated with OKT3 in the same manner. CLL1 and CLL2 are primary CLL cells from two different patients. Allogeneic T-cell targets were 7 days post OKT3 stimulation. CEM and K562 are immortalized CD19- negative leukemia cell lines. **B.** The same effector cell cultures were tested for their ability to kill allogeneic primary CLL cells. Effector cells transduced with the anti-CD19 CAR killed the CLL cells, while non-transduced T-cells from the same donor that were cultured identically did not kill CLL cells.

13.7 FIGURE 4: ANTI-CD19-TRANSDUCED T-CELLS MAINTAIN FUNCTION FOLLOWING RAPID EXPANSION

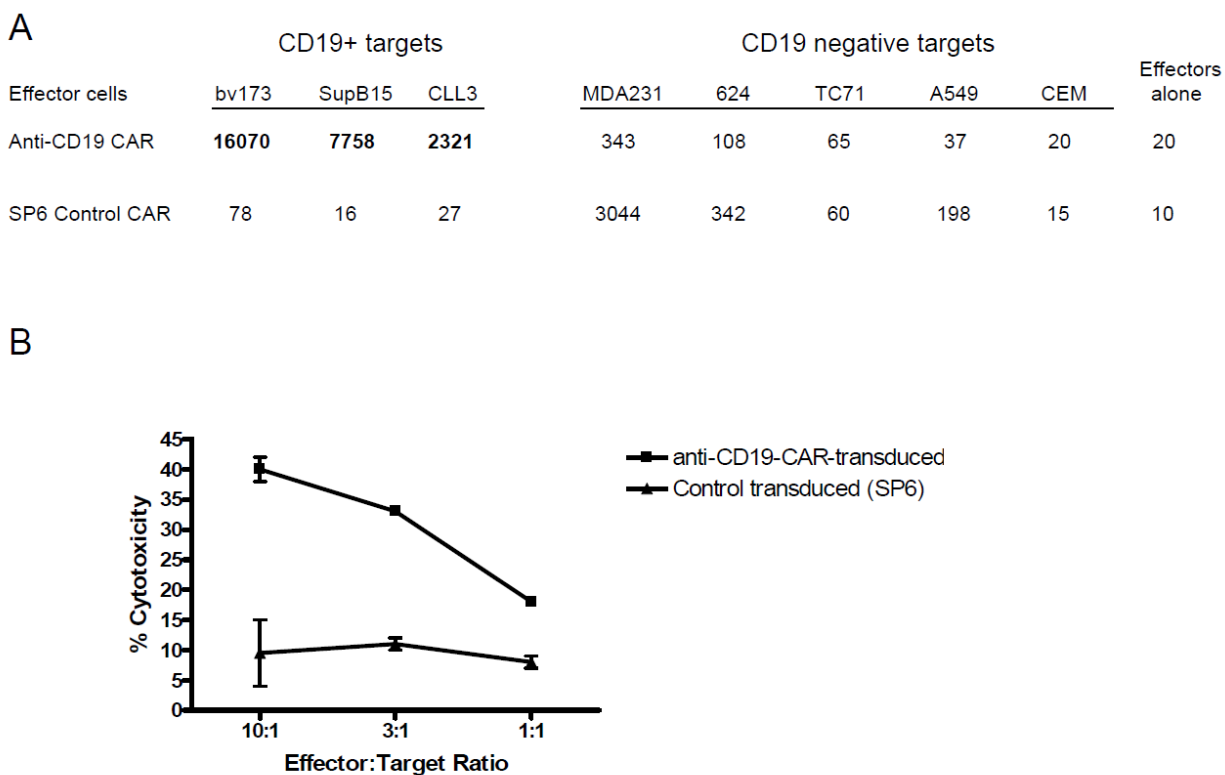


Figure 5. Following rapid expansion to a number of cells sufficient for clinical adoptive T-cell transfer, anti-CD19-transduced T-cells maintain function. A. 100,000 effector cells that were 9 days post REP initiation were cultured overnight with 100,000 target cells, and an interferon- γ ELISA was performed. All values are pg/mL of interferon- γ (mean of duplicate wells). Effector cells were either anti-CD19-CAR-transduced T-cells or T-cells transduced with the control CAR SP6 from the same patient stimulated with OKT3 in the same manner. SupB15 and bv173 are CD19-expressing leukemia cell lines, CLL3 are primary CLL cells. MDA231, A549, TC71, CEM and K562 are immortalized CD19 negative cell lines. The control CAR SP6 is specific for the chemical 2,4,6-TNP. **B** Twenty-one days after the initiation of rapid expansion, anti-CD19 CAR transduced T-cells can kill allogeneic primary CLL cells while T-cells transduced with the control CAR SP6 cannot.

13.8 FIGURE 5: T-CELLS PRODUCE IFN-GAMMA AND IL-2 IN RESPONSE TO CD-19-EXPRESSING TARGET CELLS FOLLOWING RAPID EXPANSION

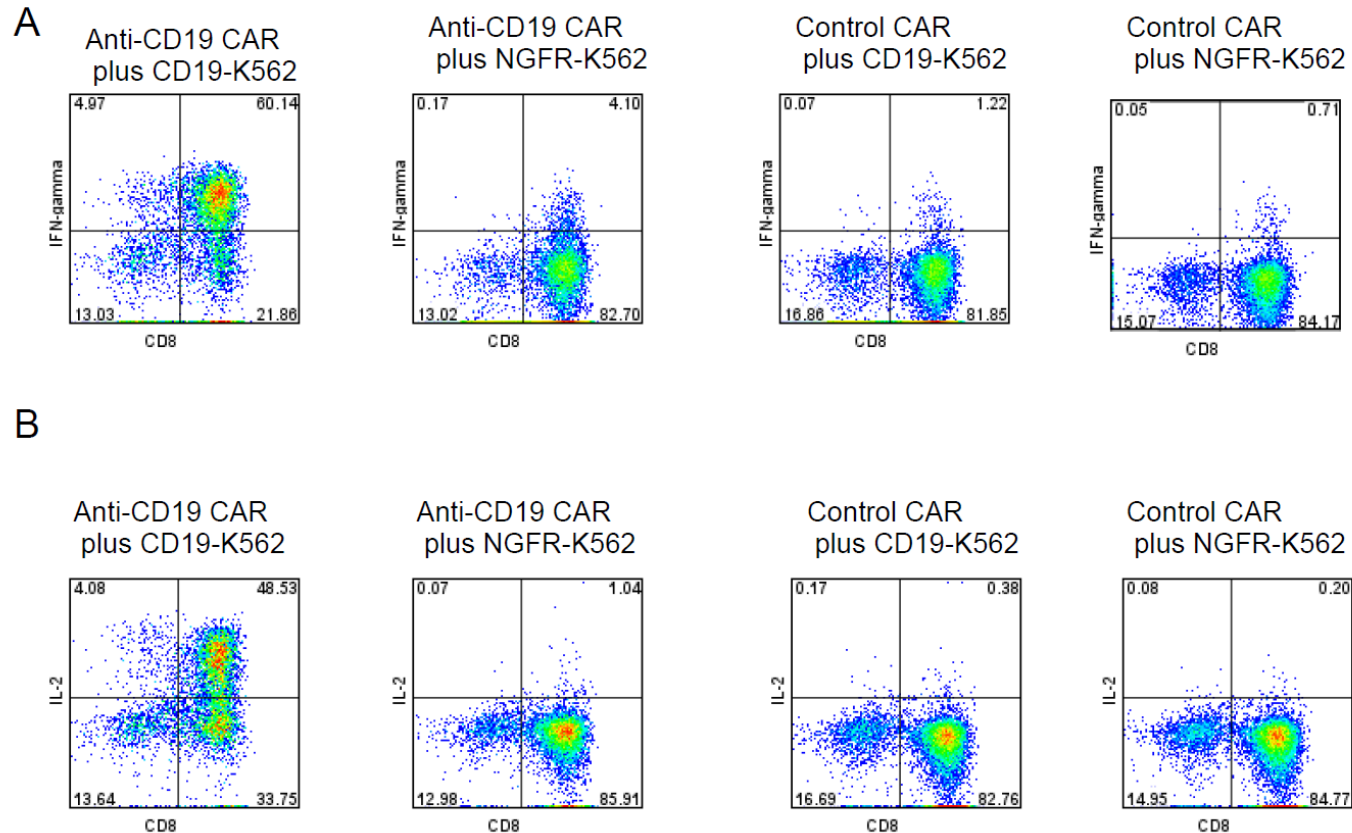
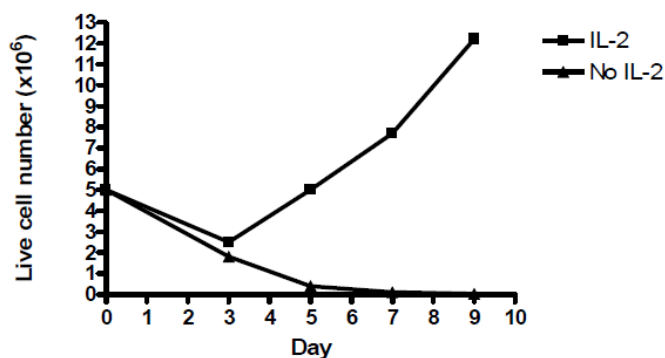


Figure 6. Fourteen days after initiation of rapid expansion a large percentage of T-cells produce IFN γ (Figure 6A) and IL-2 (Figure 6B) specifically in response to CD19-expressing target cells. Anti-CD19-CAR-transduced or control-transduced T-cells were cultured with K562 cell transduced with either full-length CD19 or nerve growth factor receptor (NGFR) for 5 hours and then intracellular cytokine staining was performed. Control-transduced T-cells were transduced with the SP6 CAR. The SP6 CAR is described in the legend for Figure 3. The K562 target cells were identical except that CD19-K562 cells were transduced with a retrovirus encoding full-length CD19 and NGFR-K562 cells were transduced with retroviruses encoding NGFR.

13.9 FIGURE 6: ANTI-CD19-CAR-TRANSDUCED T-CELLS DEPENDENT UPON EXOGENOUS IL-2 FOR IN VITRO SURVIVAL

A

Donor 1
+/- IL-2 comparison
was started on day



B

Donor 2
+/- IL-2 comparison
was started on day

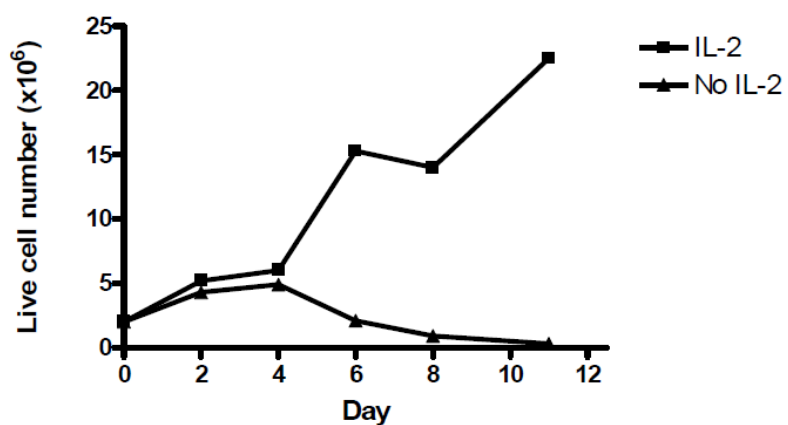


Figure 7. Anti-CD19-CAR-transduced T-cells are dependent upon exogenous IL-2 for in vitro survival. At the indicated times after initiation of a REP equal numbers of T-cells were either cultured with or without IL-2 and the number of live cells was determined by trypan blue staining on the indicated days.

13.10 FIGURE 7: OVERVIEW OF THE 6-DAY CRYOPRESERVED CELL PRODUCTION PROCESS

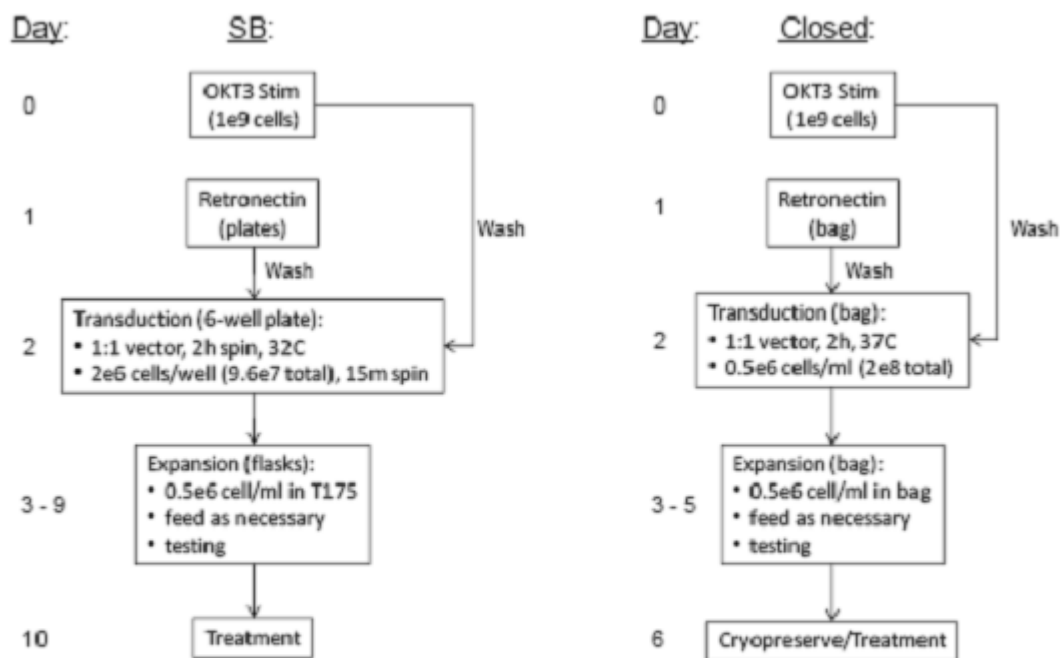


Figure 8. Overview of the 6 day cryopreserved cell production process. The schematic shows a comparison between the current 10 day Surgery Branch (SB) open cell production process to our new 6 day closed cell production process with cryopreservation.

13.11 FIGURE 8: COMPARISON OF TRANSDUCTION EFFICIENCY OF PBMC IN CLOSED VS OPEN SYSTEM

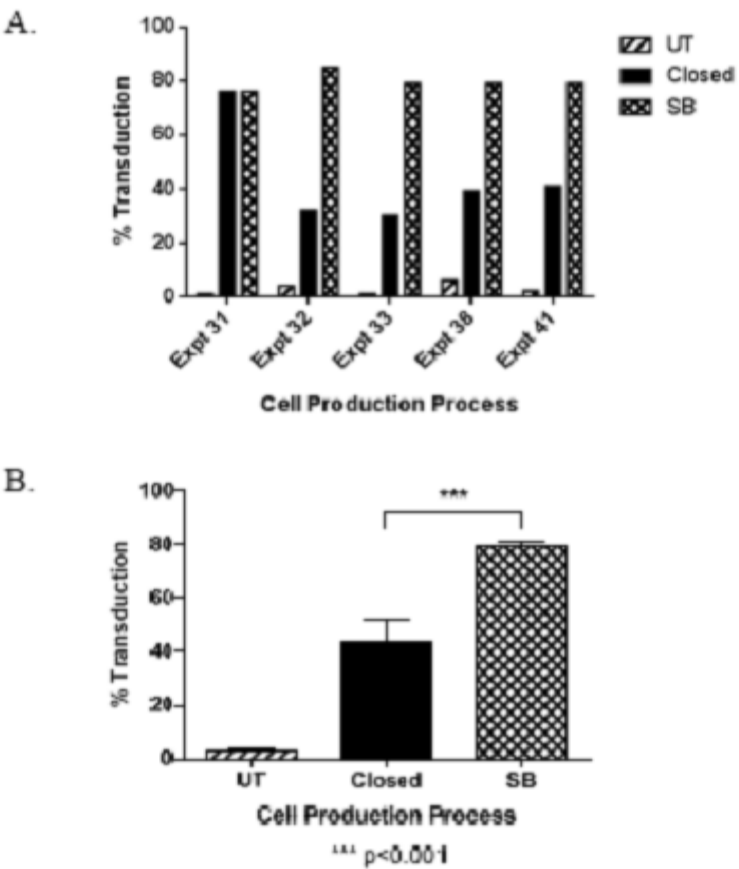


Figure 9. Comparison of transduction efficiency of PBMC in a closed versus open system. The transduction of PBMC with a Gammaretroviral vector encoding an anti-CD19 CAR in a closed bag system (black bars) was compared to The current Surgery Branch (SB) open (stippled bars) plate transduction platform. In both cases, 1×10^6 PBMC were stimulated with soluble OKT3 for 2 days, followed transduction of PBMC at a density of 0.5×10^6 cells/ml with a 1:1 diluted vector supernatant. A) In four of five experiments, transduction of PBMC in bags was significantly lower when compared to our current clinical plate transduction process (range 30 – 76%). B) Summary data for all five engineering runs at scale. Transduction in bags was significantly lower as compared to plate transductions, $43.6 \pm 8.3\%$ and $79.6 \pm 1.5\%$, $n=5$, respectively ($p < 0.001$).

13.12 FIGURE 9: TOTAL CELL NUMBER OF PBMC FOLLOWING TRANSDUCTION IN CLOSED VS OPEN SYSTEM

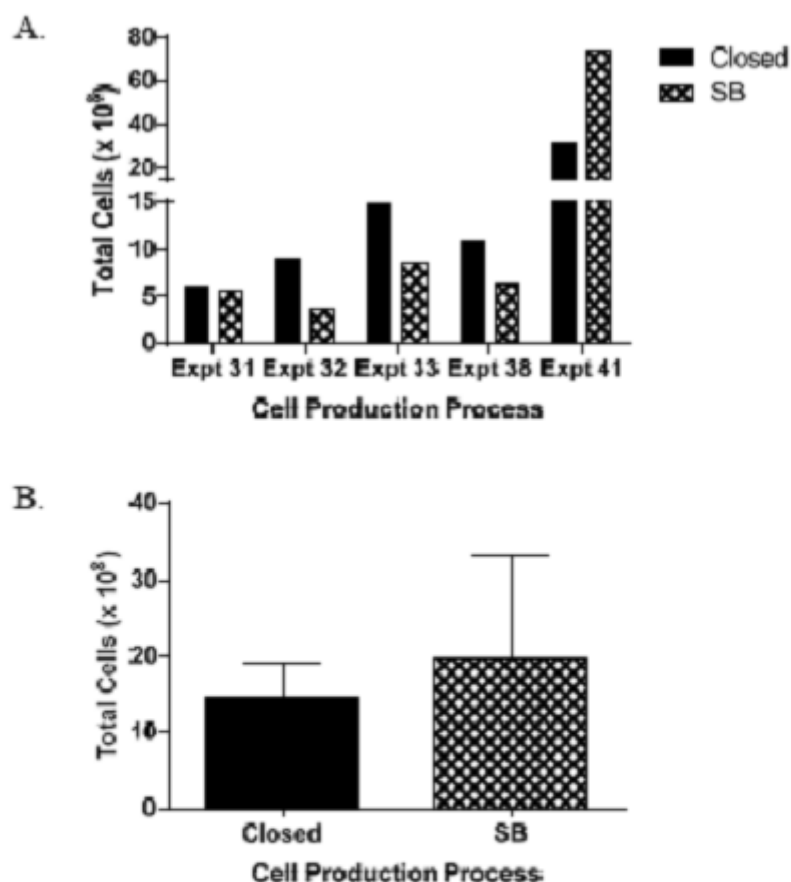


Figure 10. Total cell number of PBMC following transduction in a closed versus open system. Following PBMC transduction, the cells were washed and resuspended in cell expansion medium. For the closed process, cells were transferred into bags via sterile connection and the SB cells were expanded in upright T175 flasks. Total cell counts were calculated over 6 days for both the closed (black bars) and open (SB, stippled bars) process. A) After 6 days, total cell counts from five experiments ranged from $6.2 - 32.0 \times 10^6$ total cells and the open process range was between $3.7 - 74.0 \times 10^6$ total cells. B) There was no significant difference in total cell number when cells were expanded in the closed or open process ($14.5 \pm 4.5 \times 10^6$ and $19.6 \pm 13.6 \times 10^6$ total cells, respectively).

13.13 FIGURE 10: CELL EXPANSION OF PBMC FOLLOWING TRANSDUCTION IN CLOSED VS OPEN SYSTEM

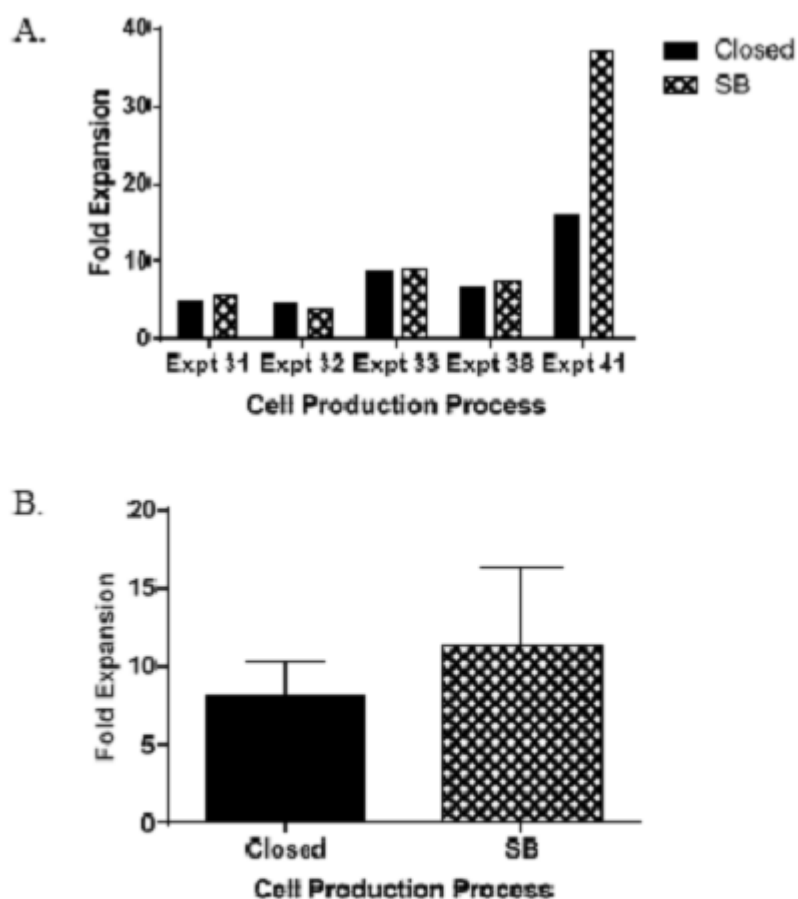


Figure 11. Cell expansion of PBMC following transduction in a closed versus open system. Following PBMC transduction, the cells were washed and resuspended in cell expansion medium. For the closed process, cells were transferred into bags via sterile connection and the SB cells were expanded in upright T175 flasks. Fold cell expansion was calculated over 6 days for both the closed (black bars) and open (SB, stippled bars) process. A) After 6 days, cells expanded the closed system in five experiments ranged from 4.5 – 16 fold as compared to 3.9 – 37 fold in the SB open process. B) There was no significant difference in total cell expansion in the closed or open process (8.2 ± 2.1 and 11.4 ± 5.0 fold expansion, respectively).

13.14 FIGURE 11: COMPARISON OF IFN-GAMMA RELEASE OF CELLS TRANSDUCED AND EXPANDED IN CLOSED VS OPEN SYSTEM

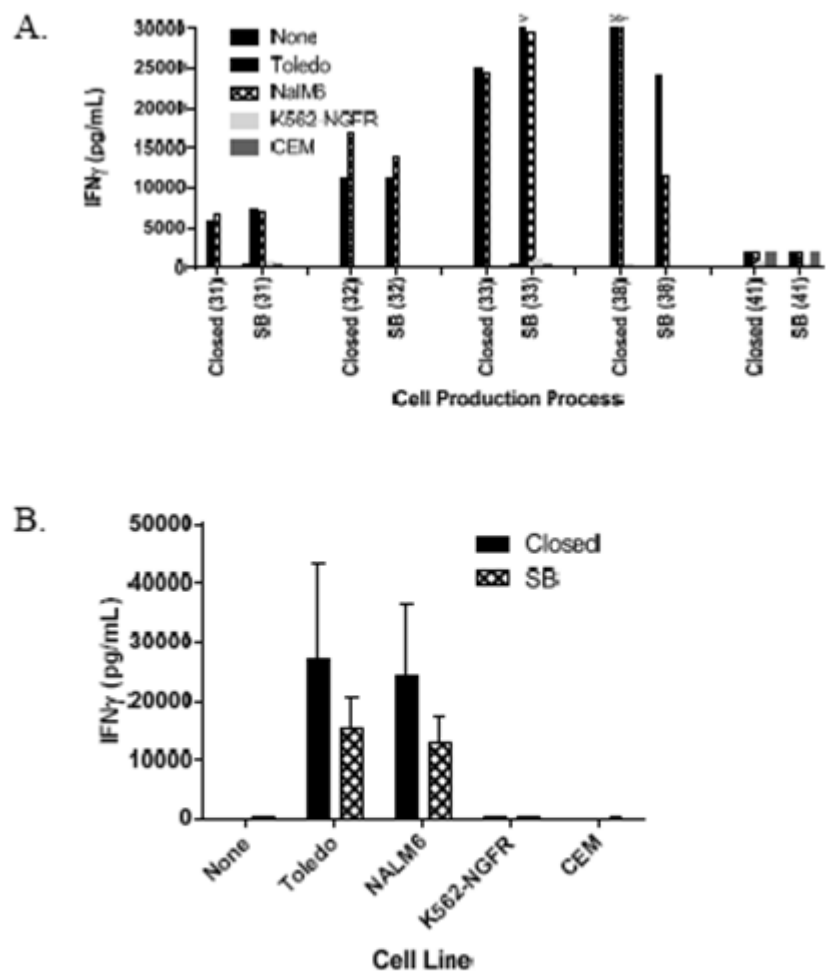


Figure 12. Comparison of IFN γ release of cells transduced and expanded in a closed versus open system. Following PBMC transduction, the cells were washed and resuspended in cell expansion medium grown for 6 days in bags (closed) or in upright T175 flasks (SB, open). IFN γ release was measured by ELISA following coculture with either CD19+ (Toledo, Nalm6) or CD19- (K562-NGFR, CEM) targets. A) IFN γ release from 5 independent experiments showing no difference in IFN γ within any single experiment. B) There was no significant difference in IFN γ release when cells were grown for 6 days in the closed or open for any of the cell lines tested.

Figure 12: Comparison of T-Cell Phenotype for PBMC Transduced and Expanded in Closed vs Open System

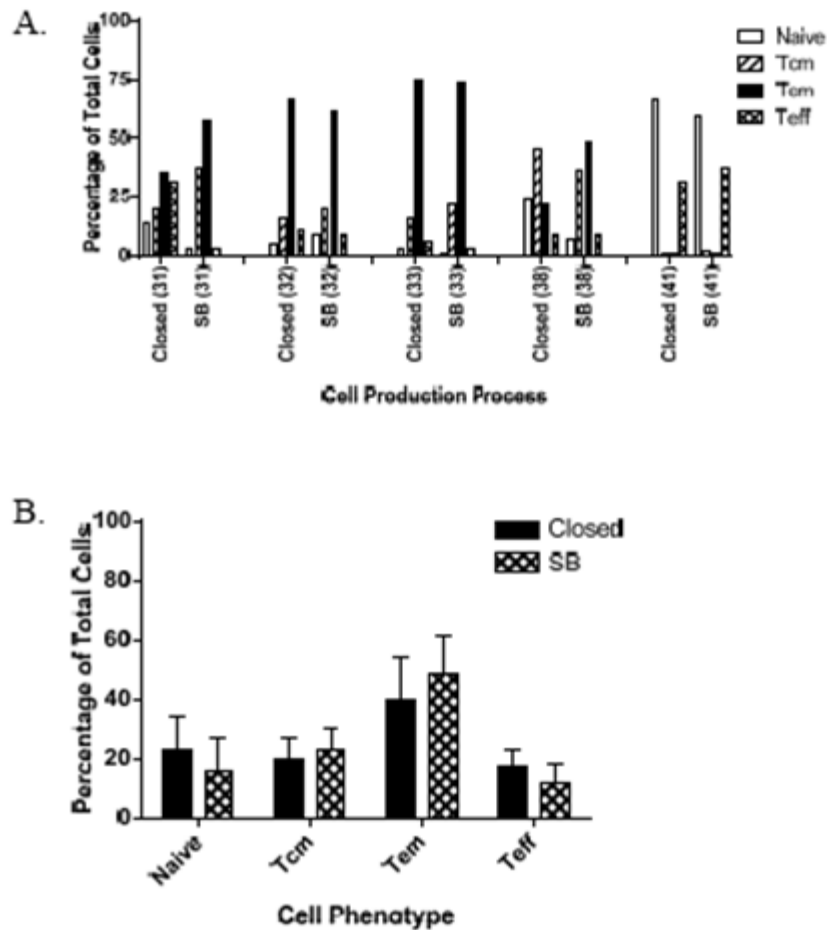


Figure 13. Comparison of T cell phenotype for PBMC transduced and expanded in a closed versus open system. Following PBMC transduction, the cells were washed and resuspended in cell expansion medium grown for 6 days in bags or in upright T175 flasks. T cell phenotype was measured by FACS following staining with anti-CD45RA and anti-CCR7 antibodies. A) T cell phenotype from 5 independent experiments was measured and showed little to no difference in phenotype within any single experiment. B) There was no significant difference in the percentage of each T cell subset measured when cells were grown for 6 days in the closed (black bars) or open (stippled bars).

13.15 FIGURE 13: COMPARISON OF PERCENTAGE AND VIABILITY OF CAR+ T-CELLS MANUFACTURED IN 6-DAY CLOSED PROCESS

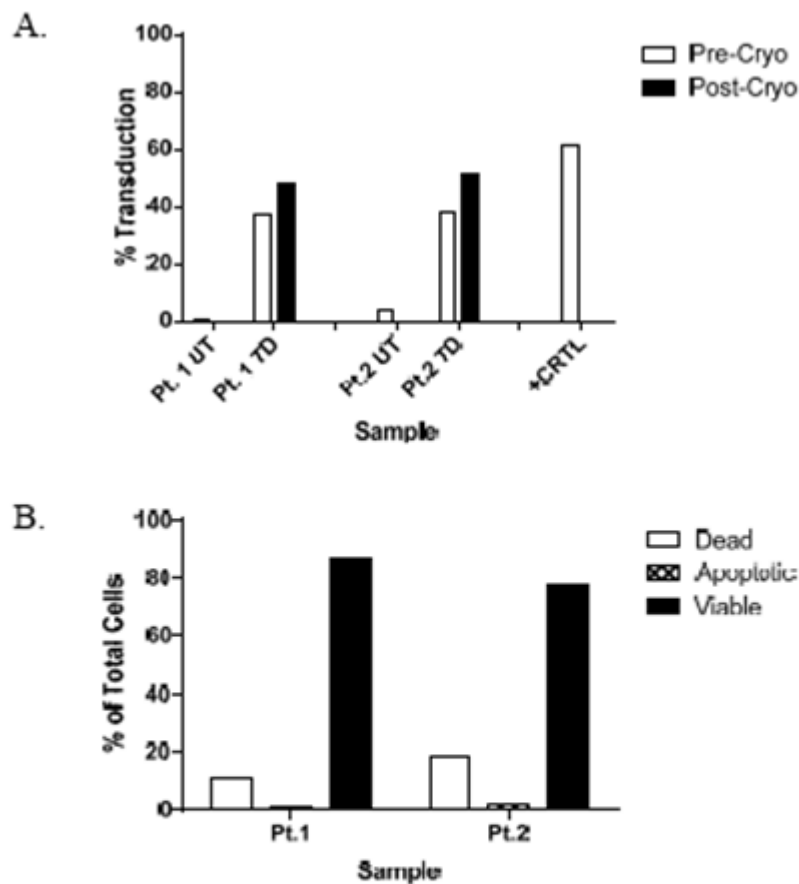


Figure 14. Comparison of the percentage and viability of CAR+ T cells manufactured in a 6 day closed process. PBMC were stimulated, transduced and expanded in bags for a total of 6 days and then cryopreserved in cryobags at a density of 8.0×10^6 cells/ml in 50ml saline-2.5% HSA-5% DMSO. A) The percentage of CD19 CAR+ T cells was measured by FACS on day 6 prior to cryopreservation and then again on day 3 post-thaw for two patients. There was no significant difference in the level of CAR+ T cells after cryopreservation (patient 1, 37.6% pre and 48.3% post; patient 2, 38.5% pre and 51.0% post). B) The viability of the cryopreserved cell product was assessed by annexin/7AAD staining. The viability of the cells at the time of thaw for patient 1 and 2 was 87% and 78%, respectively.

13.16 **FIGURE 14: COMPARISON OF IFN-GAMMA RELEASE OF CAR+ T-CELLS MANUFACTURED IN 6-DAY CLOSED PROCESS AFTER CRYOPRESERVATION**

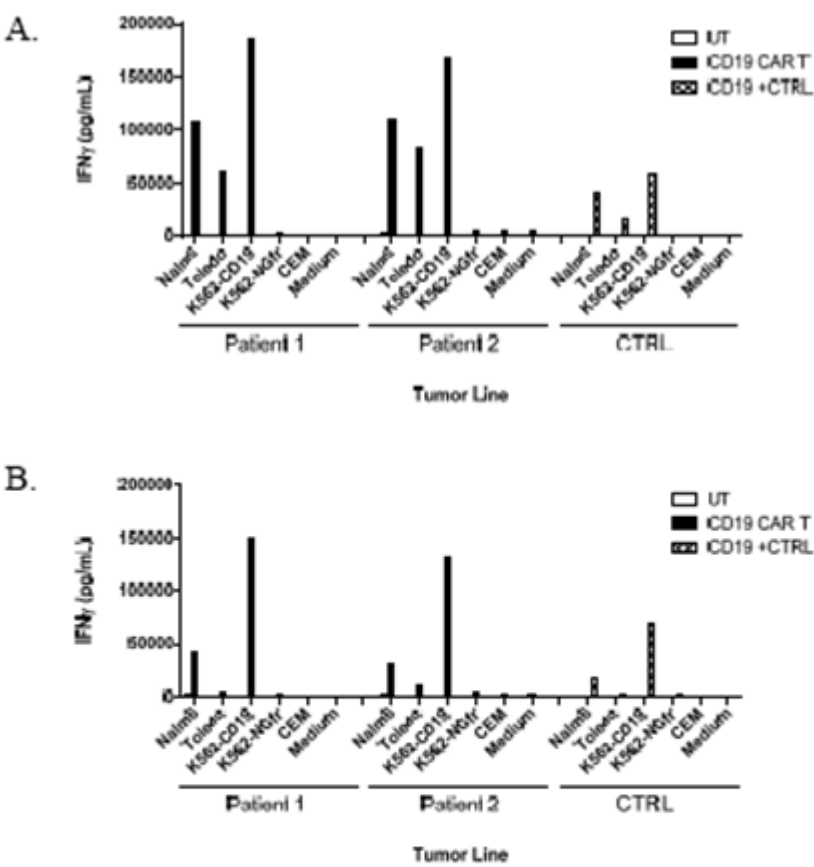


Figure 15. Comparison of IFN γ release of CAR+ T cells manufactured in a 6 day closed process after cryopreservation. PBMC were stimulated, transduced and expanded in bags for a total of 6 days and cryopreserved. After thaw (day 0), the cells were expanded in Optimizer plus 2.5% TCSR and 50 IU/ml IL-2 for 3 days. A) On day 6, prior to cryopreservation, CD19 CAR+ T cells demonstrated specific IFN γ release to CD19+ tumor targets (Nalm6, Toldedo, K562-CD19) but not to CD19- targets (K562-NGFR, CEM). B) On day 3 post-thaw, T cells were assessed for IFN γ release against CD19+ and CD19- targets. Both patients showed high levels of specific IFN γ production suggesting the cells are still CD19 CAR+ and able to recognize antigen-specific target cells.

13.17 FIGURE 15: COMPARISON OF VIABILITY AND FOLD EXPANSION OF CAR+ T-CELLS MANUFACTURED IN 6-DAY CLOSED PROCESS AFTER CRYOPRESERVATION

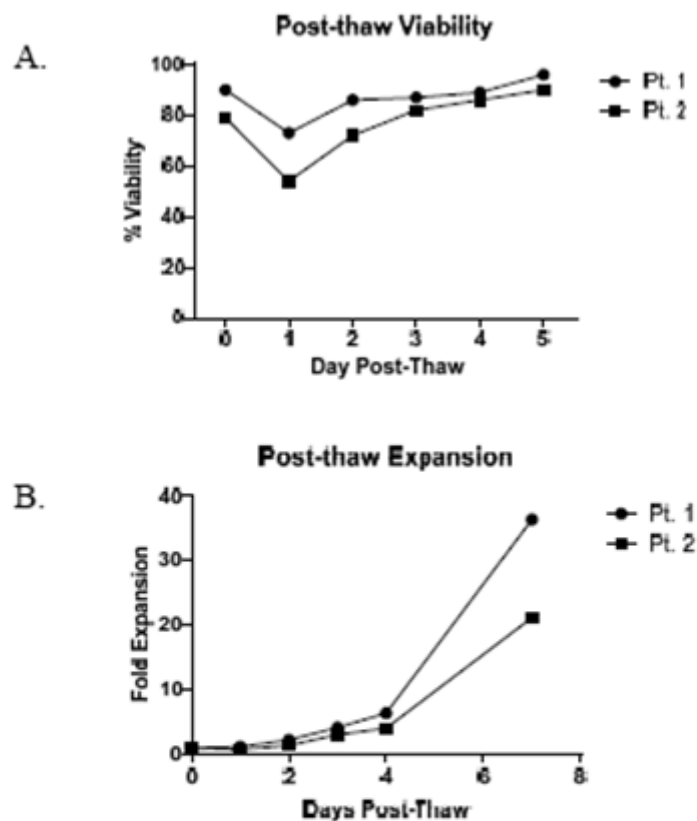


Figure 16. Comparison of the viability and fold expansion of CAR+ T cells manufactured in a 6 day closed process after cryopreservation. PBMC were stimulated, transduced and expanded in bags for a total of 6 days and cryopreserved. After thaw (day 0), the cells were washed and resuspended in Optimizer plus 2.5% TCSR and 50 IU/ml IL-2. The cells were monitored for daily for viability as well as there ability to expand post-thaw. A) Cell viability at the time of thaw was 90.0% (patient 1) and 79.9% (patient 2). A slight decrease in viability (13-15%) was observed 24h post-thaw for both patients; however cell viability recovered at 48h and continued to increase at least 90% viability over a 5 day period. B) Cell expansion post-thaw was measured. The cells expanded 36- and 21-fold, respectively, over a 7 day period.

13.18 FIGURE 16: PHENOTYPE OF CD19 CAR+ T-CELLS MANUFACTURED IN 6-DAY CLOSED PROCESS BEFORE AND AFTER CRYOPRESERVATION

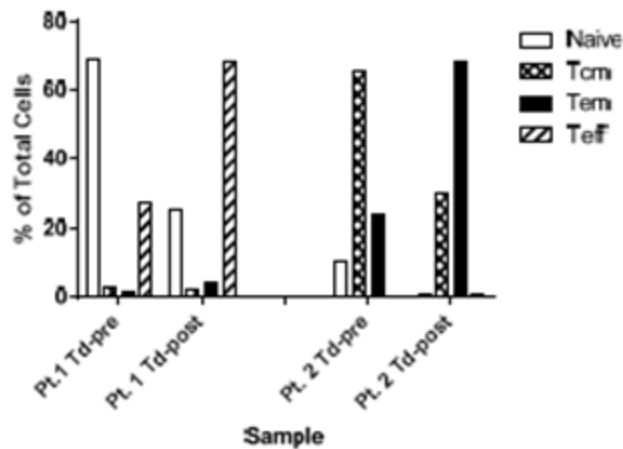


Figure 17. Phenotype of CD19 CAR+ T cells manufactured in a 6 day closed process before and after cryopreservation. PBMC were stimulated, transduced and expanded in bags for a total of 6 days and cryopreserved. After thaw (day 0), the cells were expanded in Optimizer plus 2.5% TCSR and 50 IU/ml IL-2 for 3 days. T cell phenotype was measured by FACS following staining with anti-CD45RA and anti-CCR7 antibodies. While there are slight changes in the percentages of the cell subsets represent pre- and post-cryopreservation for both patients, the overall population subsets are still represented.

13.19 APPENDIX 1: CERTIFICATE OF ANALYSIS

Anti-CD19 CAR-Transduced PBL (09-C-0082)

Date of preparation of final product:

Patient:

Tests performed on final product:

Number of days in culture:

<i>Test</i>	<i>Method</i>	<i>Limits</i>	<i>Result</i>	<i>Initials/ Date</i>
Cell viability ¹	trypan blue exclusion	>70%		
CAR positive cells/patient weight (kg)	Calculated Value	Between 0.2×10^7 and 1.0×10^7 CAR positive cells/kg		
Tumor reactivity ²	γ -IFN release vs. CD19-expressing target cell lines	>200 pg/mL		
Microbiological studies	gram stain ¹	no micro-organisms seen		
	aerobic culture ³	no growth		
	fungal culture ³	no growth		
	anaerobic culture ³	no growth		
	mycoplasma test ⁴	negative		
Endotoxin	limulus assay ¹	<5 E.U./kg		
RCR	S+L- Assay ³ RCR-PCR ⁵	negative		

¹ Performed on sample of the final product immediately prior to cryopreservation. Results are available at the time of infusion. Total viable cell number is dependent on CAR+ cells patient's weight.

² Performed 2-10 post transduction. Results are available at the time of infusion.

³ Sample collected from the final product prior to cryopreservation. Results will not be available before cells are infused into the patient.

⁴ Performed 2-10 days prior to infusion. Results are available at the time of infusion.

⁵ A sample will be saved from the final product prior to cryopreservation. If the culture period after retroviral transduction is less than 96 hours the sample will be archived. If the culture period exceeds 96 hours after retroviral transduction, the assay will be performed and results will be available at the time of cell infusion.

Prepared by: _____

Date: _____

QC sign-off: _____

Date: _____

Qualified laboratory or Clinical Supervisor

13.20 APPENDIX 2: DESCRIPTION OF DOSE DE-ESCALATIONS (COHORTS 1-7)

This trial will consist of two phases. In phase I, a dose escalation of the number of anti-CD19-CAR-transduced T-cells will be carried out. All patients in this phase of the trial will be lymphodepleted with fludarabine and cyclophosphamide, and all patients will receive aldesleukin (720,000 IU/kg every 8 hours for a maximum of 15 doses). Patients with platelet counts between 75,000 and 99,999 cells/mm³ will receive half the cyclophosphamide dose that patients with platelet counts of 100,000/mm³ or greater will receive. After a maximum tolerated cell dose is determined in phase I of the trial, phase II of the trial will begin.

Starting with approval of Amendment E (to be discontinued with approval of Amendment F), this trial is being refocused to evaluate the safety and feasibility of administering between 1.0×10^9 and 1.0×10^{10} anti CD19-CAR transduced cells and aldesleukin (720,000 IU/kg every 8 hours for a maximum of 15 doses) following a preparative conditioning regimen of fludarabine and cyclophosphamide. We have accrued 4 patients at this dose level with one patient experiencing a DLT. If we observe no more DLTs in the next 2 patients treated for a total of 1/6 patients treated, accrual will continue up to 18 patients. If at any time during accrual a cumulative total of 3 evaluable patients have experienced a DLT, then no further patients will be enrolled. If DLTs are encountered in up to 3/18 patients, the trial will be considered safe and feasible. A one-week safety assessment period will follow regimen completion (defined as the last dose of aldesleukin) between each patient in the first 6 patients and a two-week safety assessment period will follow between the 6th and 7th patient before expansion of the trial to 18 patients.

With approval of Amendment F (to be discontinued with the approval of Amendment H), the trial is being refocused to evaluate the safety and toxicity of the regimen studied at lower escalating dose levels since 3 DLTs were observed at dose level 1 (between 1.0×10^9 and 1.0×10^{10} cells). The dose of cells administered will be on a CAR positive cells/kg basis. If a DLT is observed at the revised dose levels, the dose of aldesleukin may be reduced to 72,000 IU/kg every 8 hours for a maximum of 15 doses.

Phase I – Dose Escalation (not applicable after approval of Amendment E)

The initial portion of this protocol will be a phase I dose escalation design with three cohorts of a minimum of 3 patients per cohort. The number of anti-CD19-transduced T-cells transferred for each cohort will be:

Cohort 1	between	1.0×10^9 and 1.0×10^{10}
Cohort 2	greater than	1.0×10^{10} up to 3.0×10^{10}
Cohort 3	greater than	3.0×10^{10} up to 1.0×10^{11}

Patients will be enrolled sequentially, therefore enrollment will not proceed to a higher dose level until all patients have been treated in the prior cohort. If sufficient cells cannot be grown to meet the criteria for the assigned cohort, the patient will be enrolled in the appropriate cohort for the number of cells infused. If a DLT occurs in an additional patient entered at a lower dose due to cell growth limitations, accrual will continue at this level as described in the dose-escalation scheme below. Accrual will be halted at the higher level until the cohort at the lower level is complete as described below.

Should a single patient experience a dose limiting toxicity at a particular dose level, three more patients would be treated at that dose to confirm that no greater than 1/6 patients have a DLT prior to proceeding to the next higher level. If a level with 2 or more DLTs in 3-6 patients has been identified, three additional patients will be accrued at the next-lowest dose for a total of 6, in order to further characterize the safety of the maximum tolerated dose prior to starting the phase II portion of the trial. After the maximum tolerated dose is determined, the phase II portion of the trial will begin.

A one-week safety assessment period will follow regimen completion (defined as the last dose of aldesleukin) between each patient in a cohort except for the last patient in each cohort for which a two-week safety assessment period will follow regimen completion (defined as the last dose of aldesleukin) before a patient will be accrued to the next cohort.

Upon completion of the Phase I portion of the study, the adverse events observed in this cohort will be reported to and evaluated by the IRB before proceeding to the Phase II portion.

Phase II – Randomization to Chemotherapy Versus No Chemotherapy (not applicable after approval of Amendment E)

In phase II of the trial, patients will be randomized to two treatment arms. Patients assigned treatment arm 1 will receive fludarabine and cyclophosphamide lymphodepleting chemotherapy prior to receiving the engineered PBL cells while patients assigned to treatment arm 2 will not receive chemotherapy. Randomization will also be stratified so that an equal number of patients with circulating malignant cells (leukemia) will be entered on each arm of the phase II part of the trial.

One to four days after completion of chemotherapy, anti-CD19 CAR-transduced T-cells will be infused. After cell infusion, IV aldesleukin therapy will be initiated (720,000 IU/kg every 8 hours for a maximum of 15 doses). The randomization performed during phase II of this trial will be stratified so that each arm will receive an equal number of patients with a total peripheral blood CD3 count greater than or equal to 500 cells/microliter, and each arm will receive an equal number of patients with a CD3 count less than 500 cells/microliter. In addition, the randomization will be stratified so that each arm will receive an equal number of patients with peripheral blood involvement with malignant cells. Patients with peripheral blood involvement with malignant cells are defined as patients with pre-treatment peripheral blood lymphocyte counts greater than 4000 lymphocytes per microliters.

During both phases multiple immunologic assays will be carried out on peripheral blood T-cells to determine persistence of anti-CD19-CAR-transduced T-cells as well as their function. During phase II an assessment of the anti-malignancy effect of anti-CD19-CAR-transduced T-cells will be made. All patients will be followed for anti-malignancy response, survival and toxicity.

Dose Escalation and Cohort Expansion (with approval of Amendment F, not applicable after approval of Amendment H)

With approval of Amendment F, the trial is being refocused to evaluate the safety and toxicity of the regimen studied at lower escalating dose levels since 3 DLTs were observed at dose level 1 (between 1.0×10^9 and 1.0×10^{10} cells). For cohort 2, 3 patients will be treated with NMA, 0.5×10^7 CAR positive cells/kg ($\pm 20\%$), and high dose aldesleukin (720,000 IU/kg). This starting dose of cells has been selected due to PK modeling of interferon gamma levels and toxicity data from the first 8 patients treated on this study. If one DLT is observed in a patient treated in Cohort 2, after

analysis of the IFN-gamma cytokine data, 3 additional patients will be treated at this dose level (0.5×10^7 CAR positive cells/kg ($\pm 20\%$) after receiving NMA followed by a reduced dose of aldesleukin (72,000 IU/kg every 8 hours for a maximum of 15 doses) (Cohort 3). This 10-fold reduction of aldesleukin was previously investigated in a study of patients with renal cancer. The incidence of grade 3 and 4 aldesleukin related toxicities was less in patients treated with 72,000 IU/kg every 8 hours than those treated with the higher dose of 720,000 IU/kg aldesleukin, every 8 hours (Yang, J.C., et al., J Clin Oncol. 2003, 21(16):3127-32).

If no DLTs are observed in the 3 patients in Cohort 2, after analysis of the IFN-gamma cytokine data, 3 additional patients will be treated with NMA, 1.0×10^7 CAR positive cells/kg ($\pm 20\%$) and high dose aldesleukin (Cohort 4). If one DLT is observed in a patient treated in Cohort 4, 3 additional patients will be treated at this dose level (1.0×10^7 CAR positive cells/kg ($\pm 20\%$) after receiving NMA followed by a reduced dose of aldesleukin (72,000 IU/kg every 8 hours for a maximum of 15 doses) (Cohort 5).

Cohort	Treatment Regimen		
	<i>NMA Chemotherapy Regimen (as specified in protocol)</i>	<i>Cell Dose</i>	<i>IL-2 Dose</i>
1 (Closed with Amendment F)	Yes	between 1.0×10^9 and 1.0×10^{10} cells	High dose (720,000 IU/kg)
2	Yes	0.5×10^7 CAR positive cells/kg ($\pm 20\%$)	High dose (720,000 IU/kg)
3 ¹	Yes	0.5×10^7 CAR positive cells/kg ($\pm 20\%$)	Low dose (72,000 IU/kg)
4	Yes	1.0×10^7 CAR positive cells/kg ($\pm 20\%$)	High dose (720,000 IU/kg)
5 ²	Yes	1.0×10^7 CAR positive cells/kg ($\pm 20\%$)	Low dose (72,000 IU/kg)

¹ Cohort 3 will be skipped if there are no DLTs in Cohort 2.

² Cohort 5 will be skipped if there are no DLTs in Cohort 4.

The highest dose cohort evaluated with no DLTs in 3 patients (either 3, 4 or 5) will be expanded to a total of 18 evaluable patients, subject to not exceeding 3 patients in total with a DLT.

Safety Assessment

Cytokine (IFN-gamma levels) and toxicity data must be analyzed prior to dose escalation. A one-week safety assessment period will follow regimen completion (defined as the last dose of aldesleukin) between each patient in a cohort except for the last patient in each cohort for which a two-week safety assessment period will follow regimen completion (defined as the last dose of aldesleukin) before a patient will be accrued to the next cohort. A two-week safety assessment period will follow between the 3th and 4th patient before expansion of the trial to 18 patients.

Dose Escalation and Cohort Expansion (with approval of Amendment H, closed with Amendment J)

With Amendment H, cohorts 2-5 will be closed and two new cohorts will be opened at the same cell dose level as cohorts 2 and 4 but without aldesleukin. These cohorts will be designated cohort 6 and 7. For Cohort 6, 3 patients will be treated with NMA, and 0.5×10^7 CAR positive cells/kg ($\pm 20\%$). If one DLT is observed in a patient treated in Cohort 6, after analysis of the IFN-gamma cytokine data, accrual will be halted pending discussions with the FDA and IRB.

In November 2011, the first patient in Cohort 6 experienced a DLT of grade 3 confusion (possible aphasia), and grade 3 neuropathy (facial droop) possibly related to the cell therapy. The serum IFN-gamma levels were low in this patient (range 9-44 pg/mL) following treatment with cells. These events were discussed with the FDA, and the FDA indicated that we can treat an additional patient in Cohort 6 (with approval of Amendment I). If this additional patient does not experience a DLT, Cohort 6 will be expanded to a total of 6 patients. If any additional patients treated in Cohort 6 experiences a DLT, after analysis of the IFN-gamma cytokine data, accrual will be halted pending discussions with the FDA and IRB.

If no additional DLTs are observed in the 6 patients in Cohort 6, after analysis of the IFN-gamma cytokine data, 3 patients will be treated with NMA, and 1.0×10^7 CAR positive cells/kg ($\pm 20\%$) in Cohort 7. If one DLT is observed in a patient treated in Cohort 7, 3 additional patients will be accrued to Cohort 7. If 2 DLTs occur in 6 or less patients in Cohort 7, no additional patients will be accrued at this dose level.

Cohort	Treatment Regimen		
	<i>NMA Chemotherapy Regimen (as specified in protocol)</i>	<i>Cell Dose</i>	<i>IL-2 Dose</i>
1 (Closed with Amendment F)	Yes	between 1.0×10^9 and 1.0×10^{10} cells	High dose (720,000 IU/kg)
2 (Closed with Amendment H)	Yes	0.5×10^7 CAR positive cells/kg ($\pm 20\%$)	High dose (720,000 IU/kg)
3 (Closed with Amendment H)	Yes	0.5×10^7 CAR positive cells/kg ($\pm 20\%$)	Low dose (72,000 IU/kg)
4 (Closed with Amendment H)	Yes	1.0×10^7 CAR positive cells/kg ($\pm 20\%$)	High dose (720,000 IU/kg)
5 (Closed with Amendment H)	Yes	1.0×10^7 CAR positive cells/kg ($\pm 20\%$)	Low dose (72,000 IU/kg)
6 (Closed with Amendment J)	Yes	0.5×10^7 CAR positive cells/kg ($\pm 20\%$)	None
7 (Closed with Amendment J)	Yes	1.0×10^7 CAR positive cells/kg ($\pm 20\%$)	None

The highest dose cohort evaluated with no DLTs in 3 or more patients, or 1 DLT in 6 patients will be expanded to a total of 18 evaluable patients, subject to not exceeding 3 patients in total with a DLT.

Safety Assessment

Cytokine (IFN-gamma levels) and toxicity data must be analyzed prior to dose escalation. A one-week safety assessment period will follow regimen completion between each patient in a cohort except for the last patient in each cohort for which a two-week safety assessment period will follow regimen completion before a patient will be accrued to the next cohort. A two-week safety assessment period will follow the last patient treated in the dose escalation cohort before expansion of the trial to 18 patients.

Dose De-escalation and Cohort Expansion

In February 2012, the second patient in Cohort 6 experienced DLTs. The SAEs consisted of grade 3 hypotension and grade 3 confusion (possibly aphasia) related to the cell therapy. These toxicities have resolved and the patient has been discharged. After discussions with the FDA, cohort 6 and 7 were closed and two new dose de-escalation cohorts were opened as follows:

13.21 APPENDIX 3: DESCRIPTION OF DOSE ESCALATION FOR FRESH ANTI-CD19 TIL (COHORTS 8-10)

PBMC will be obtained by leukapheresis (approximately 1.0×10^{10} cells). In most patients, whole PBMC will be cultured in the presence of anti-CD3 (OKT3) and aldesleukin in order to stimulate T-cell growth. In some patients the peripheral blood might be contaminated with large numbers of CD19-expressing malignant cells. In patients with peripheral blood lymphocytes counts greater than 4000 cells per microliter, CD19-expressing cells will be depleted using the Miltenyi Biotec CliniMacs system with anti-CD19 microbeads and then CD19-depleted PBMC will be stimulated with OKT3. In all patients, 2 days after OKT3 stimulation transduction will be initiated by exposure of approximately 1.0×10^7 to 5.0×10^8 cells to supernatant containing the anti-CD19 CAR retroviral vector. These transduced cells will be expanded and tested for their anti-tumor activity. Successful TCR gene transfer will be determined by FACS analysis for the CAR and specific anti-tumor reactivity will be tested by cytokine release measured against CD19+ cell lines and CD19-negative cell lines. Successful CAR gene transfer for each transduced PBL population will be defined as >30% CAR positive cells and for biological activity, gamma-interferon secretion against CD19 targets must be at least 200 pg/mL. The cells administered vary depending on their growth characteristic. In prior protocols over 3.0×10^{11} T-cells have been safely infused to cancer patients.

Due to toxicities experienced during the dose escalation phase of this trial, the protocol has been amended several times to decrease the dose and eliminate the administration of IL-2. The description of these changes has been moved to [Appendix 2](#).

Beginning with Amendment M patients will be lymphodepleted with fludarabine and a reduced dose of cyclophosphamide – 30mg/kg/day for 2 days and will receive 1.0×10^6 CAR positive cells/kg ($\pm 20\%$) as noted below.

Cohort	Treatment Regimen		
	<i>NMA Chemotherapy Regimen (as specified in protocol)</i>	<i>Cell Dose</i>	<i>IL-2 Dose</i>
8 (De-escalation Dose 1)	Yes	2.5×10^6 CAR positive cells/kg ($\pm 20\%$)	None
9 (De-escalation Dose 2)	Yes	1.0×10^6 CAR positive cells/kg ($\pm 20\%$)	None
10 (De-escalation Dose 2)	Yes – dose of both agents reduced	1.0×10^6 CAR positive cells/kg ($\pm 20\%$)	None

Cohort 8 will initially accrue 3 patients. If no DLTs are observed in these 3 patients, three additional patients will be accrued. If DLTs are observed in ≤ 1 patient out of 6 patients treated, this cohort will be expanded as described below. If a DLT is observed in one of the first 3 patients, after analysis of the cytokine data, 3 additional patients can be accrued to this cohort. If more than 1 out of 6 patients in this cohort experience a DLT, this cohort will be closed, and patients will be accrued to cohort 9. Cohort 8 was closed with Amendment M.

Cohort 9 will initially accrue 3 patients. If no DLTs are observed in these 3 patients, three additional patients will be accrued. If DLTs are observed in ≤ 1 patient out of 6 patients treated,

this cohort will be expanded as described below. If a DLT is observed in one of the first 3 patients, after analysis of the cytokine data, 3 additional patients can be accrued to this cohort. If more than 1 out of 6 patients in this cohort experience a DLT, this cohort will be closed. Subsequent dose reductions will be discussed with the FDA prior to initiation. If ≤ 1 patient in 6 patients treated develops a DLT then the 12 remaining patients will be enrolled in cohort 10 for a total of 18 evaluable patients at a dose level of 1.0×10^6 CAR positive cells/kg ($\pm 20\%$), subject to not exceeding 3 patients in total with a DLT.

Following administration of the cell product, neurological status will be closely monitored and urgently managed as described in Section 3.4.3.

In addition, we will be evaluating stored serum samples in an effort to identify the particular cytokines which may be contributing to these toxicities.

Safety Assessment

Cytokine (IFN-gamma levels) and toxicity data must be analyzed prior to dose de-escalation. A one-week safety assessment period will follow regimen completion between each patient in a cohort except for the last patient in each cohort for which a two-week safety assessment period will follow regimen completion before a patient will be accrued to the next cohort. Beginning with Amendment R, the one week safety assessment period between patients will no longer be required during the expansion phase of the protocol for the remaining patients to be enrolled in Cohort 10.

The study will be halted pending discussions with the FDA and IRB if the following conditions are met:

Prior to Amendment E

- If two DLTs occur in the first cohort of the Phase I portion of this study.
- Two or more patients develop a grade 3 or greater toxicity at any point in the study not attributable to the chemotherapy preparative regimen (or circumstances unrelated to this study).
- If 1 of the first 3 patients (OR 2 of the first 6 patients, OR 3 of the first 9 patients, OR 4 of the first 12 patients) develop DLT due to autoimmune toxicity.
- Development of EBV lymphoma or polyclonal lymphoproliferative disease (PLPD) in an EBV negative subject on this or any other SB adoptive cell therapy study. Accrual of EBV negative subjects will be halted only.

With Approval of Amendment E (not applicable with approval of Amendment F)

- If two DLTs occur in the first 6 patients treated on this study.
- Two or more patients develop a grade 3 or greater toxicity at any point in the study not attributable to the chemotherapy preparative regimen (or circumstances unrelated to this study).
- If 1 of the first 3 patients (OR 2 of the first 6 patients, OR 3 of the first 9 patients, OR 4 of the first 12 patients) develop DLT due to autoimmune toxicity.

- Development of EBV lymphoma or polyclonal lymphoproliferative disease (PLPD) in an EBV negative subject on this or any other SB adoptive cell therapy study. Accrual of EBV negative subjects will be halted only.

With Approval of Amendments F, H, and J

As stated above, the study will be halted pending discussions with the FDA and IRB if the following conditions are met:

- If cohorts 2-7 or 9 cannot be expanded due to DLTs.
- During the expansion phase, if at any time during accrual, a cumulative total of 3 evaluable patients have experienced a DLT.
- Two or more patients develop a grade 3 or greater toxicity at any point in the study not attributable to the chemotherapy preparative (or circumstances unrelated to this study).
- If 1 of the first 3 patients (OR 2 of the first 6 patients, OR 3 of the first 9 patients, OR 4 of the first 12 patients) in cohorts 2-5 develop DLT due to autoimmune toxicity.
- Development of EBV lymphoma or polyclonal lymphoproliferative disease (PLPD) in an EBV negative subject on this or any other SB adoptive cell therapy study. Accrual of EBV negative subjects will be halted only.

13.22 APPENDIX 4: MODIFICATION OF DOSE CALCULATIONS* IN PATIENTS WHOSE BMI IS GREATER THAN 35

Beginning with Amendment M, actual body weight will be used for dose calculations of all treatment agents.

1. BMI Determination:

$$\text{BMI} = \text{weight (kg)} / [\text{height (m)}]^2$$

2. Calculation of ideal body weight:

$$\text{Male} = 50 \text{ kg} + 2.3 (\text{number of inches over 60 inches})$$

Example: Ideal body weight of 5'10" male

$$50 + 2.3 (10) = 73 \text{ kg}$$

$$\text{Female} = 45.5 \text{ kg} + 2.3 (\text{number of inches over 60 inches})$$

Example: Ideal body weight of 5'3" female

$$45.5 + 2.3 (3) = 57 \text{ kg}$$

3. Calculation of "practical weight":

Calculate the average of the actual and the ideal body weights. This is the practical weight to be used in calculating the doses of chemotherapy and associated agents designated in the protocol.

*Practical weight will NOT be used in the calculation of dose for aldesleukin.