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PROTOCOL TITLE

Phase I/II Study of Metastatic Cancer Using Lymphodepleting Conditioning Followed by Infusion of Anti-VEGFR2 Gene Engineered CD8+ Lymphocytes

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Drug Name:	anti-VEGFR2 CAR CD8+ peripheral blood lymphocytes (PBL)
IND Number:	IND 14440
Sponsor:	Steven A. Rosenberg, M.D., Ph.D.

Précis:

Background:

- We have constructed a single retroviral vector that contains a chimeric T cell receptor (CAR) that recognizes the Vascular Endothelial Growth Factor Receptor 2 (VEGFR2), which can be used to mediate genetic transfer of this CAR with high efficiency (> 50%) without the need to perform any selection. Administration of VEGFR2 CAR transduced cells inhibited tumor growth in several different models in different mouse strains.
- In co-cultures with VEGFR2 expressing cells, anti-VEGFR2 transduced T cells secreted significant amounts of IFN-γ□with high specificity.

Objectives:

Primary objectives:

- To evaluate the safety of the administration of anti-VEGFR2 –CAR engineered CD8+ peripheral blood lymphocytes in patients receiving a non- myeloablative conditioning regimen, and aldesleukin.
- Determine if the administration of anti-VEGFR2 –CAR engineered CD8+ peripheral blood lymphocytes and aldesleukin to patients following a nonmyeloablative but lymphoid depleting preparative regimen will result in clinical tumor regression in patients with metastatic cancer.

Secondary objective:

- Determine the in vivo survival of CAR gene-engineered cells.

Eligibility:

Patients who are 18 years of age or older must have

- metastatic cancer;
- previously received and have been a non-responder to or recurred after standard care for metastatic disease;

Patients may not have:

- contraindications for high dose aldesleukin administration.

Design:

- PBMC obtained by leukapheresis (approximately 5 X 10^9 cells) will be cultured in the presence of anti-CD3 (OKT3) and aldesleukin in order to stimulate T-cell growth.
- Transduction is initiated by exposure of approximately 10^8 to 5 X 10^8 cells to retroviral vector supernatant containing the VEGFR2 genes.
- Patients will receive a nonmyeloablative but lymphocyte depleting preparative regimen consisting of cyclophosphamide and fludarabine followed by intravenous infusion of ex vivo CAR gene-transduced CD8+ PBMC plus IV aldesleukin. With approval of amendment C, aldesleukin (based on total body weight) will be administered at a dose of 72,000 IU/kg as an intravenous bolus over a 15 minute period approximately every eight hours (+/- 1 hour) beginning within 24 hours of the cell infusion and continuing for up to 5 days (maximum 15 doses
- Patients will undergo complete evaluation of tumor with physical examination, CT of the chest, abdomen and pelvis and clinical laboratory evaluation four to six weeks after treatment. If the patient has SD or tumor shrinkage, repeat complete evaluations will be performed every 1-3

months. After the first year, patients continuing to respond will continue to be followed with this evaluation every 3-4 months until off study criteria are met.

- The study will be conducted using a Phase I/II optimal design. The protocol will proceed in a phase 1 dose escalation design. Initially, the protocol will enroll 1 patient in each dose cohort unless that patient experiences a dose limiting toxicity (DLT). Should a single patient experience a dose limiting toxicity due to the cell transfer at a particular dose level, additional 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. If a dose limiting toxicity occurs in the first cohort, that cohort will be expanded to 6 patients. If 2 DLTs are encountered in this cohort, the study will be terminated. If IFN-gamma levels increase substantially (as defined in the protocol) in the patient in a cohort compared to the prior patient, the cohort would be expanded to an n=3 to obtain more data on this phenomenon. If one of these 3 patients experience a DLT, the cohort will be expanded to six patients. Following amendment C, patients will be enrolled in cohorts 8-11, with the non-myeloablative chemotherapy regimen, cells and low dose aldesleukin following a conventional 3+3 design. Once the MTD has been determined, the study then would proceed to the phase II portion. Patients will be entered into two cohorts based on histology: cohort 1 will include patients with metastatic melanoma and renal cancer, and cohort 2 will include patients with other types of metastatic cancer.
- For each of the 2 strata evaluated, the study will be conducted using a phase II optimal design where initially 21 evaluable patients will be enrolled. For each of these two arms of the trial, if 0 or 1 of the 21 patients experiences a clinical response, then no further patients will be enrolled but if 2 or more of the first 21 evaluable patients enrolled have a clinical response, then accrual will continue until a total of 41 evaluable patients have been enrolled in that stratum.
- The objective will be to determine if the combination of aldesleukin, lymphocyte depleting chemotherapy, and anti-VEGFR2 CAR-gene engineered CD8+ lymphocytes is able to be associated with a clinical response rate that can rule out 5% (p0=0.05) in favor of a modest 20% PR + CR rate (p1=0.20).

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1.0 Introduction

1.1. Study Objectives:

1.1.1. Primary objectives:

- To evaluate the safety of the administration of anti-VEGFR2 –CAR engineered CD8+ peripheral blood lymphocytes in patients receiving the non-myeloablative conditioning regimen, and aldesleukin
- Determine if the administration of anti-VEGFR2 CAR-engineered CD8+ peripheral blood lymphocytes and aldesleukin to patients following a nonmyeloablative but lymphoid depleting preparative regimen will result in clinical tumor regression in patients with metastatic cancer.

1.1.2. Secondary objective:

- Determine the in vivo survival of CAR gene-engineered cells.

1.2. Background and Rationale:

Studies in experimental animals have demonstrated that the cellular rather than the humoral arm of the immune response plays the major role in the elimination of murine tumors. Much of this evidence was derived from studies in which the adoptive transfer of T lymphocytes from immune animals could transfer resistance to tumor challenge or in some experiments, the elimination of established cancer. Thus, most strategies for the immunotherapy of patients with cancer have been directed at stimulating strong T cell immune reactions against tumor-associated antigens.

In contrast to antibodies that recognize epitopes on intact proteins, T cells recognize short peptide fragments (8-18 amino acids) that are presented on surface class I or II major histocompatibility (MHC) molecules and it has been shown that tumor antigens are presented and recognized by T cells in this fashion. The molecule that recognizes these peptide fragments is the T-cell receptor (TCR). The TCR is analogous to the antibody immunoglobulin molecule in that, two separate proteins (the TCR alpha and beta chains) are brought together to form the functional TCR molecule. An alternate approach to enable T cells to recognize targets is to utilize a chimeric T cell receptor which is constructed by using the variable regions of the heavy and light chains of an antibody connected by a linker sequence and fused to CD28, CD3 zeta and 41BB signaling chains. Thus the T cell can recognize and signal based on the non-MHC restricted recognition of the target. The goal of this protocol is to transfer genes encoding a chimeric receptor that recognizes VEGFR2 on tumor vasculature into normal peripheral blood lymphocytes (PBL) derived from cancer patients and to return these engineered cells to patients aimed at mediating regression of their tumors. This trial is similar to previous Surgery Branch TCR gene transfer adoptive immunotherapy protocols except that we will use a receptor that recognizes targets based on the recognition of an antibody reactive with the VEGFR2 molecule on the tumor vasculature. This has the potential to treat patients with common epithelial malignancies of multiple histologies.

1.2.1. Prior Surgery Branch Trials of Cell Transfer Therapy Using Tumor Infiltrating Lymphocytes in Patients with Metastatic Melanoma

Studies in the Surgery Branch, National Cancer Institute, identified genes that encode melanoma tumor associated antigens (TAA) recognized by tumor infiltrating lymphocytes (TIL) in the context of multiple MHC class I molecules[1]. These TAA appeared to be clinically relevant antigens responsible for mediating tumor regression in patients with advanced melanoma since the TIL used to identify these antigens were often capable of mediating in vivo anti-tumor regression. Two antigens, which were present in virtually all fresh and cultured melanomas, were called MART-1 (Melanoma Antigen Recognized by T Cells - 1), and gp100. The genes encoding these two antigens have been cloned and sequenced. The MART-1 gene encodes a 118 amino acid protein of 13 kd. The gp100 gene encodes a protein identical to that recognized by monoclonal antibody HMB-45. These antigens were thus the original targets of our gene therapy cell transfer protocols in patients with metastatic melanoma.

In the great majority of murine models demonstrating the therapeutic effectiveness of the adoptive transfer of lymphocytes mediating tumor regression, immunosuppression of the host prior to the adoptive transfer of lymphocytes was required. North and colleagues demonstrated that adoptive transfer of lymphocytes was not effective unless the mouse was immunosuppressed by total body irradiation or chemotherapy prior to adoptive transfer of lymphocytes[2]. Very similar observations were made in other rodent cancer models[3]. We thus incorporated a lymphodepleting chemotherapy into our human cell transfer studies.

In the Surgery Branch, NCI, we developed a protocol to rapidly expand heterogeneous TILs for adoptive transfer[4]. TILs were expanded using the rapid expansion protocol (REP) in the presence of OKT3, irradiated allogeneic feeder cells and IL-2. These REPed TILs retained highly specific in vitro anti-tumor activity, often contained reactivities against several antigenic epitopes and contained both CD8+ and CD4+ lymphocytes. These autologous bulk TIL were re-infused to patients following a nonmyeloablative chemotherapy with cyclophosphamide and fludarabine. These patients subsequently received high-dose IL-2 and some received peptide immunization when the TIL reactivity was against known MART-1 and gp100 peptides (protocol 99-C-0158). A total of 43 patients received this treatment[5,6]. This regimen resulted in objective cancer regressions in 49% of patients (21 of 43) with metastatic melanoma (Table 1).

Murine models predicted that increasing the extent of lymphodepletion could increase the effectiveness of the cell transfer therapy[7]. Thus, we performed two additional sequential trials of ACT with autologous anti-tumor lymphocytes (TIL) in patients with metastatic melanoma. Increasing intensity of host preparative lymphodepletion consisting of cyclophosphamide and fludarabine with either 200cGy (25 patients) or 1200 cGy (25 patients) total body irradiation (TBI) was administered prior to cell transfer. While non-myeloablative chemotherapy alone showed an objective response rate of 49%, when 200cGy or 1200cGy TBI was added the response rates were 52% and 72% respectively (Table 1). TBI appeared to result in increased patient survival. Responses were seen in all visceral sites including brain. Host lymphodepletion was associated with increased serum levels of the lymphocyte homeostatic cytokines IL-7 and IL-15[7]. Objective responses were correlated with the telomere length of the transferred cells[8].

Patients exhibited the expected hematological toxicities associated with the cyclophosphamide, fludarabine and TBI preparative regimens. Other toxicities are shown in Table 2. Patients recovered marrow function rapidly after cell infusion with absolute

neutrophil counts greater than 500 per mm^3 by day 12 and sustained platelet counts above 20,000 per mm^3 by day 14 (except 4 patients on the TBI 1200 protocol with platelet recovery on days 16, 17, 20, and 22).

1.2.2. Surgery Branch Trials of Cell Transfer Therapy Using Transduction of Anti-TAA TCR Genes into Non-reactive TIL or PBL

It is often not possible to isolate sufficient tumor samples from melanoma patients and even when tumor is available, only about 60 to 70% generate melanoma reactive TIL cultures. As a potential alternative to the requirement to establish TIL cultures from melanoma patients, we sought methods that could be used to easily obtain a polyclonal population of T cells with anti-TAA properties. Transfer of antigen specific TCR genes to PBL is a potential method generating large numbers of reactive anti-cancer T cells[9]. In a murine model of this approach, TCR gene transfer into murine splenocyte T-cells was performed using a retroviral vector[10]. The engineered T cells were shown to expand in vivo upon viral challenge and efficiently homed to effector sites. In addition, small numbers of TCR-transduced T cells promoted the rejection of antigen-expressing tumors in the mice. 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[11],[12].

To expand on and potentially improve on these early results, we next isolated TCR genes from both gp100 and MART-1 reactive T cells. These studies have recently been published and are presented briefly here[11],[12]. To test the in vivo efficacy of these MART-1 TCR engineered T cells, 31 HLA-A*0201 patients with progressive metastatic melanoma were treated. Results in the first 17 patients were published in 2006 [13]. All patients were refractory to prior therapy with IL-2. T cell cultures from all patients were biologically reactive, with specific secretion of interferon-γ following co-culture with either MART-1 peptide pulsed T2 cells and or melanoma cell lines expressing the MART-1 antigen. Gene transfer efficiencies measured by staining for Vβ12 expression in these lymphocytes ranged from 17% to 67% (mean value 42%). Four of the 31 patients demonstrated a sustained objective regression of their metastatic melanoma assessed by standard RECIST criteria.

There were no toxicities in any patient attributed to the gene-marked cells. We thus demonstrated for the first time in humans, that normal autologous T lymphocytes, transduced *ex vivo* with anti-TAA TCR genes and reinfused in cancer patients can persist and express the transgene long-term *in vi*vo and mediate the durable regression of large established tumors.

A similar study was conducted using gp100 TCR gene marked cells, however this retroviral vector had a low titer when produced under GMP conditions. Fourteen patients were treated on this study. No antitumor responses have been seen. There have been no grade 5 toxicities observed on this study, and all grade 3 and 4 toxicities observed were expected toxicities associated with the non-myeloabalative chemotherapy regimen or IL-2.

The low response rate in our prior MART-1 TCR gene transfer protocol led us to identify MART-1 reactive TCR with higher avidity than the MART-1 F4 TCR used in the prior gene therapy clinical trial[14], [15]. We have now treated 24 patients with metastatic melanoma using autologous PBL transduced with an improved MART-1 F5 TCR following a non-myeolablative chemotherapy. Six patients (25%) have achieved an objective partial response. Toxicities were similar to those seen in the prior TCR gene

therapy trial except that 15 patients developed a transient mild anterior uveitis easily reversed by steroid eye drops and ten patients developed decreased hearing reversed by middle ear steroid injections. Transient rashes have also been seen. There were no treatment related deaths.

We have also conducted a clinical trial with a TCR that recognizes the gp100:154-162 melanoma peptide. This TCR was raised in an HLA-A2 transgenic mouse immunized with this peptide[15]. We have now treated 21 patients with metastatic melanoma using autologous PBL transduced with this improved gp100 TCR following a non-myeloablative chemotherapy. Four patients (19%), have achieved an objective partial response. Seven patients developed a transient mild anterior uveitis reversed by steroid eye drops and ten patients developed decreased hearing reversed by middle ear steroid injections. There were no treatment related deaths.

In addition to the studies listed above in patients with metastatic melanoma, we have recently initiated several studies for patients with other metastatic cancers using PBL transduced with TCR genes or chimeric antibody receptor (CAR)genes. We have studied PBL transduced with TCR genes targeting p53, CEA, NY-ESO-1 and TRAIL bound to the DR4 Receptor, and PBL transduced with CAR targeting Her-2 and CD19. Patients on these studies also received a non-myeloablative chemotherapy regimen consisting of cyclophosphamide and fludarabine, and high dose IL-2. In two studies, 08-C-0121 (anti - ESO-1 TCR) and 09-C-0082 (anti-CD19 CAR), we have seen impressive clinical response. The clinical responses observed in seven of the fifteen evaluable patient receiving anti-ESO TCR-engineered peripheral blood lymphocytes have been very encouraging. Of the six synovial cell sarcoma, four patients have confirmed PRs. Of the nine patients with highly ESO expressing melanoma, there have been 2 confirmed complete responses and 2 confirmed partial responses. In study 09-C-0082, an impressive ongoing partial remission at 7 months post treatment of the lymphoma occurred in the first patient treated on this study. This patient had heavily pre-treated, progressive follicular lymphoma that involved all major lymph node areas. Only three small lesions that were consistent with lymphoma persisted on positron emission tomography (PET) imaging post-treatment.

Most toxicities observed in these studies were expected toxicities of the chemotherapy and aldesleukin administration. However, in 2 studies, we have observed serious adverse events related to the transduced cells. In 09-C-0051 (anti-Her2 CAR transduced PBL), the first patient, with metastatic colorectal cancer, was treated with 10^{10} autologous T cells transduced with the retrovirus encoding an anti-Her-2 CAR. This patient developed respiratory distress and died four days later. This toxicity was apparently due to a previously unrecognized ability of this CAR to recognize Her-2 expressed on lung epithelial cells although the exact explanation for the toxicity is not clear. In 09-C-0047 (anti-CEA TCR transduced PBL), all three patients treated experienced a variety of gastrointestinal events which were attributed to the gene/cell therapy including diarrhea, and colitis. All gastrointestinal events have since resolved in these patients, and the patients' colonic mucosa has returned to normal, and the patients have normal bowel function. Grade 3 diarrhea lasting longer than 72 hours is considered a DLT per protocol and this event was observed in two of three patients enrolled in protocol 09-C-0047, meeting the criteria for stopping protocol accrual.

1.2.3. VEGFR2 as a Target for Cell Transfer Studies

As discussed above, adoptive cell transfer (ACT) immunotherapy strategies designed to directly target antigens expressed on tumor cells can result in durable objective

regression in patients with metastatic melanoma. Despite its demonstrated effectiveness in experimental mouse tumor models and in the human, the application of ACT is limited because of the need to identify antigens with highly selective expression in cancer. Antiangiogenic approaches can potentially have wide application to a variety of tumor types, though the cytostatic nature of the available treatments and the redundancy of angiogenic pathways have limited its effectiveness. Redirecting immune cells to target tumor vasculature can combine the advantages of cell transfer and antiangiogenic approaches and offers an alternative to overcome the obstacles confronting conventional tumor-specific immunotherapy and the use of cytostatic antiangiogenic inhibitors.

Most solid tumors and some hematologic malignancies are characterized by an angiogenic phenotype which is an absolute requirement for their survival, progression, and metastasis[16,17,18,19,20,21]. Therapeutic approaches targeting molecules involved in tumor angiogenesis can inhibit tumor growth.

Proliferating endothelial cells in the vessels within solid tumors aberrantly express high levels of angiogenic growth factors, receptors and adhesion molecules that are absent or barely detectable in established blood vessels, which are normally quiescent. Among these, vascular endothelial growth factor (VEGF) and its receptors appear to be the regulators of angiogenesis responsible for the vascularization of normal and neoplastic tissues[21,22,23] . Over expression of VEGF and its receptors (VEGFR) are strongly associated with tumor angiogenesis, survival, invasion, metastasis, recurrence, and prognosis in human cancers. VEGF stimulates angiogenesis mainly through VEGFR receptor-2 (known as flk1 in mouse and KDR in human), a tyrosine kinase receptor that is overly expressed in tumor endothelial cells and on some tumor cells[24,25,26,27]. Pharmacologic approaches to inhibit VEGF or VEGFR using monoclonal antibodies or small molecules are of value in cancer treatment though the cytostatic nature of these interventions and the redundancy of angiogenic pathways have limited the curative potential of these treatments.

Several immunotherapeutic approaches targeting VEGFR-2 on endothelial cells have been used to inhibit pathological angiogenesis and tumor growth, which include neutralization of VEGFR-2[28], immunization against VEGFR-2 or coupling VEGF to toxins to target and destroy VEGFR-2 positive cells[29] as well as disruption of VEGFR genes though thus far these approaches have been of limited clinical value.

Chimeric T cell receptors utilize the variable regions of an antibody as a single chain attached to the intracellular signaling chains of a conventional T cell receptor to produce a molecule that when transduced into lymphocytes enables the cell to recognize targets based on the antigen recognition of the antibody. The availability of monoclonal antibodies against VEGFR2 and the antitumor potency of ACT suggested to us that the transduction of a chimeric receptor recognizing VEGFR2 into lymphocytes could produce self-replicating T cells capable of selectively destroying tumor vasculature.

1.2.4. Preclinical Studies to Provide the Rationale for this Clinical Protocol

We generated a series of MSGV-based recombinant retroviral vectors encoding a chimeric antigen receptor comprised of the single chain variable regions (ScFv) of an anti-mouse VEGFR-2 antibody DC101 linked to the intracellular mouse T cell signaling sequences derived from the mouse CD28 and 4-1BB molecules, and CD3ς chains of the T cell receptor via the mouse CD8α hinge and transmembrane regions (referred as DC101-CD828BBZ). We also constructed a retroviral vector lacking the 4-1BB signaling domain (referred to as DC101-CD828Z), a vector which retains DC101 ScFv sequence but lacked

the intracellular T cell signaling sequences (referred as DC101-CD8), a vector encoding a ScFv directed against a synthetic hapten TNP (termed SP6-CAR) and a vector lacking any of the CAR sequences (termed empty vector). All of these recombinant retroviral constructs described are schematically represented in Figure 1A.

Surface expression of the retrovirally encoded transgene products in transduced $CD3^+$ primary mouse T cells was determined by flow cytometry. As shown in Figure 1B, the DC101 CAR expressing vectors efficiently and consistently transduced ConA/IL-7 activated mouse T cells (range 79-86%), which were mostly (~90%) $CD8^+$ at 5 days post transduction (Fig. 1C). The intensity of CAR expression derived from the DC101 CAR comprising the 4-1BB signaling moiety was consistently lower than that derived from vectors lacking 4-1BB sequence in all the transduction experiments (n = 5). We then assessed the ability of DC101-CAR engineered T cells to respond to immobilized VEGFR-2 protein. While there were no differences between T cells transduced with various vectors to respond to immobilized anti-mouse CD3 antibody, only the T cells engineered with DC101-CAR containing intact intracellular signaling sequences responded specifically to plate bound target VEGFR-2 protein, as measured by proliferation (Fig. 1D) and IFN-γ secretion (Fig. 1E).

Next we determined whether DC101 CAR modified T cells could specifically recognize mouse cell lines expressing VEGFR-2. Mouse endothelial cell lines and tumor lines from various tissue origins were examined for cell surface expression of VEGFR-2 by flow cytometry. The level of VEGFR-2 varied among the cell lines tested. While all the transformed mouse endothelial cell lines (SVEC4-10EHR1, bEND-3, SVR, and MS1) showed high levels of VEGFR-2 expression, most of the tumor lines showed either low (MC38, 4T1, P815, and MCA205), or undetectable levels (MC17-51, EL-4, C4198, B16-F10, MB49, and NIH-3T3) of cell surface VEGFR-2 protein (Fig. 2A). Two of the VEGFR-2 negative cell lines MB49 and NIH-3T3 were stably transduced with a lentiviral vector expressing VEGFR-2 (termed MB49-Flk1 and 3T3Flk1) and were used as positive controls in subsequent *in vitro* T cell functional assays.

A representative experiment showing the MHC nonrestricted IFN-γ response of mouse T cells engineered to express DC101 CARs is presented in Figure 2B. IFN-γ response was specifically detected in response to most of the VEGFR-2 positive cell lines tested and the amount of IFN-γ secretion was highly correlated with the level of VEGFR-2 expressed on target cells (Fig. 2B inset). Reactivity was restricted to T cells expressing DC101-CAR containing intact T cell signaling domains. There were no significant differences in the performance of DC101-CAR with or without 4-1BB signaling sequences.

To determine the therapeutic efficacy of our tumor vasculature targeting strategy, DC101 CAR transduced cells were used to treat mice bearing five different established vascularized subcutaneous tumors. Mice received 2 daily doses of exogenous rhIL-2 for 3 days beginning with the first day of T cell therapy.

Results presented in Figure 3A demonstrate that T cells expressing the DC101 CAR were capable of mediating a significant growth inhibitory effect against the poorly immunogenic B16-F10 (B16) melanoma, the MC38 colon cancer, the MCA-205 sarcoma in C57BL/6 mice and the CT26 colon and Renca renal cancer in BALB/C mice. There was no antitumor effect in mice treated with empty vector transduced T cells or IL-2 alone. T cells expressing DC101 ScFv but lacking the intracellular T cell signaling molecules (Fig. 3C) or the transfer of recombinant DC101 antibody at this dose (Fig. 3A) had no effect on controlling the growth of established B16 tumor. Additionally, mice treated with

T cells engineered with an irrelevant CAR (SP6 CAR) or an empty vector failed to induce tumor inhibition. The treatment effect of DC101-CAR endowed T cells against the B16 melanoma was a direct function of the number of cells administered (Fig. 3D). Delay in tumor growth could be achieved with as low as $2x10^6$ DC101 CAR transduced T cells (*P*= 0.008). While, DC101 CAR transduced T cells on their own self evoked a moderate antitumor effect in the absence of exogenous IL-2, their tumor inhibitory effect was significantly enhanced by rhIL-2 coadministration (P= 0.009) (Fig.4A).

T cells transduced with DC101 CAR vector containing the 4-1BB intracellular signaling sequences (DC101-CD828BBZ) or lacking those sequences (DC101-CD28Z) performed equally well and were statistically indistinguishable (*P*= 0.1) in delaying the growth of established bulky tumors (Fig. 4A). Further, T cells transduced with either of the vectors was significantly enhanced by exogenous IL-2 administration (*P* = 0.004, 0.009 respectively).

To define whether 4-1BB signaling could improve the long-term persistence of CAR modified T cells *in vivo*, tumor from two mice from each of the treatment groups that received DC101 CAR engineered T cells and rhIL-2 (from experiment shown in Fig. 4A) were harvested at 30 days post T cell transfer and individually analyzed for the presence of DC101 CAR expressing T cells by flow cytometry (Fig. 4B). Mice treated with DC101-CD828BBZ CAR engineered T cells had 4-5 fold more DC101 CAR expressing $CD3^+$ in the tumor than mice treated with T cells carrying DC101-CD828Z CAR that lacked 4-1BB. These results suggested that 4-1BB influenced the persistence of adoptively transferred antigen-specific T cells at the tumor site.

Trafficking of effector T cells to the tumor site is one of the important factors limiting an effective antitumor treatment of adoptively transferred T cells. To determine the extent and kinetics of trafficking of DC101 CAR T cells to tumor, we harvested tumors and spleens from mice treated with either empty vector or DC101 CAR transduced Thy1.1T cells at various times afer adoptive therapy and performed flow cytometric analysis. The percentage of infused T cells that specifically expressed Thy1.1 in the total population of single cell preparations of spleen and the lymphocyte gated population of cells from tumor was determined. Representative FACS data (Fig. 5A) and pooled data obtained from three different mice from independent experiments (Fig. 5B) are shown. By day 3 adoptively transferred T cells trafficked similarly to spleen and tumor irrespective of their genetic modification. However, at day 6 and 9, trafficking to the tumor was far greater for the DC101 CAR endowed T cells.

We also examined the toxicity resulting from the administration of CAR expressing VEGFR-2. No signs of morbidity or mortality were seen in C57BL/6 mice treated with VEGFR-2 CAR transduced syngeneic T cells under conditions that mediated significant anti-tumor effects. Histopathologic analysis of various organs in C57BL/6 mice treated with maximum numbers (2×10^7) of VEGFR-2 CAR modified T cells showed no evidence of treatment related toxicity, despite reports of low levels of VEGFR-2 expression in vascularized tissues such as kidney, retina, and pancreas. In contrast, severe toxicity was seen in tumor bearing BALB/c mice treated similarly with 2×10^7 DC101-CAR transduced syngeneic T cells. However, in BALB/c mice comparable tumor inhibitory effect was achieved with minimal morbidity if the number of administered T cells was reduced to 5×10^6. Histopathologic analysis of BALB/c mice treated with 2×10^7 DC101-CAR transduced T cells and IL-2 revealed findings characteristic of cytokine induced hypotension including multifocal mild coagulation necrosis in the liver and mild hepatic pericholangitis and pulmonary perivasculitis, villous atrophy, villous blunting, and crypt

epithelial hyperplasia of the small intestine and colon. No abnormalities in the gross appearance of the heart, lungs, liver, kidneys, spleen, pancreas, uterus, ovaries, or brain were seen.

1.2.5. Construction and testing of retroviral vectors expressing CAR against human KDR

The ability of the T cell based antivascular targeting strategy to treat established, poorly immunogenic vascularized tumors in mouse tumor models led us to explore the translation of our preclinical findings to the treatment of human tumors. We constructed a set of recombinant retroviral vectors expressing CAR against the human VEGFR-2 (KDR) using the sequences from the 1121 monoclonal antibody from Imclone Corp.[30] The first KDR CAR vector termed KDR1121-hCD828BBZ was comprised of a ScFv derived from a fully human antibody KDR1121(*54*) specific to the human KDR antigen linked to the hinge and transmembrane sequences from the human CD8α, which was in turn fused to the intracellular sequences derived from the human CD28, 4-1BB, and CD3ς chain of TCR. A second vector referred to as KDR1121-hCD28Z was composed of KDR1121 ScFv linked to the hinge and transmembrane sequences and the intracellular signaling domain of the human CD28 molecule followed by cytoplasmic sequences from the CD3ς chain of TCR. The configuration and components of each of these vectors are illustrated in Figure 6A.

The functional integrity of these KDR CAR encoding retroviral vectors was tested *in vitro* in several assays. Supernatants from gibbon ape leukemia virus-pseudotyped high titer virus producer cells were used to transduce OKT3 activated human PBLs from 3 different donors. The KDR CAR encoding retroviral vectors transduced human PBLs at a high frequency resulting in 79 to 85% of the $CD3^+$ T cells expressing the CAR on the cell surface (Fig. 6B). Even though both vectors could transduce human PBLs at a comparable rate, the MFI of CAR expression driven from KDR-CD828BBZ CAR vector possessing the 4-1BB signaling sequence was slightly lower (Figure 6B and C), a result similar to our findings with the DC101 CAR in mouse T cells (Fig. 1C). However, no differences were seen between these two KDR CAR vectors transduced into human T cells when measuring antigen-specific proliferation (Fig. 6D) and IFN-γ secretion (Fig. 6E) when the cells were exposed to immobilized KDR protein *in vitro.*

Next we tested the ability of KDR CAR modified T cells to recognize KDR positive human cells as measured by specific IFN-g secretion. KDR1121-CD828BBZ and KDR1121-CD28Z CAR transduced PBLs from 3 different donors, 8 days post transduction were cocultured for 24 hours with KDR negative 293 cells or 293-KDR cells, a stable transfectant expressing KDR protein (Fig. 6G). Data presented in Figure 6F indicated that both the vectors conferred similar levels of specificity and functionality to the transduced human T cells as evidenced from their ability to specifically recognize only the KDR expressing 293-KDR cells and not the 293 cells.

We also determined the activity of KDR1121-CD828BBZ CAR transduced T cells against various primary human cells. A panel of normal primary human endothelial and epithelial cells from different tissue origins and muscle myoblasts, cultured *in vitro* for a short period, were examined for KDR expression by flow cytometry. The KDR protein was readily detectable only in cultured primary endothelial cells and not in any of the primary epithelial cells and myoblasts tested (Fig. 6G). The intensity of KDR expression was higher in skin-derived human dermal microvascular endothelial cells (HMVEC-D) compared to lung-derived HMVEC (HMVEC-L) or HUVECs. Notably, the KDR CAR

transduced T cells secreted IFN-γ only in response to KDR positive endothelial cells irrespective of their tissue origin and failed to recognize the other primary cells tested (Fig. 6H).

Thus based on these experiments we selected the KDR1121-hCD828BBZ vector for use in our human clinical protocols.

The preclinical studies provides a rationale for the translation of VEGFR2 CAR transduced cells for the treatment of human cancer.

1.2.6. 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 non-myeloablative chemotherapy used in this protocol has been administered to over 150 patients and all have reconstituted their hematopoietic systems[7].

CAR transduced human T cells has been administered to humans in several clinical trials. We treated 14 patients with metastatic ovarian cancer utilizing autologous T cells transduced with a retroviral vector encoding a single chain antibody that recognized the ovarian cancer-associated antigen alpha-folate receptor. The single chain antibody was linked to the signaling domain of the Fc receptor gamma chain. These 14 patients received up to 1.7×10^{11} cells intravenously and except for mild malaise and fevers there were no toxicities attributed to the cell administration. Lamers et al. reported on the treatment of three patients who received autologous T cells transduced with a retrovirus encoding an antibody against the carbonic anhydrase IX molecule expressed on renal cell cancers. Three patients were treated and mild transaminase elevations precluded the treatment of additional patients. This was probably due to the expression of carbonic anhydrase IX expression on biliary ductal epithelium.

Pule, et al. engineered human T cells to express a CAR directed to the diasialoganglioside GD2, a tumor associated antigen expressed by human neuroblastoma cells[31]. These CAR transduced cells were administered to eleven children between the ages of 3 and 10, all of whom had metastatic neuroblastoma. There were no adverse effects attributable to the genetically modified CAR T cells in the eleven subjects that were followed for up to two years following cell infusion. Two patients exhibited an objective response and several additional patients exhibited necrosis in established tumors. Persistence of the transduced cells was greater when the CAR were inserted into EBV specific cytolytic T lymphocytes than when the CAR were inserted into T cells that were activated by an anti-CD3 monoclonal antibody.

Till et al. reported the adoptive immunotherapy of patients with indolent non-Hodgkin's lymphomas and mantle cell lymphoma using specific T cells transfected with CAR recognizing the CD20 molecule. Seven patients were treated and one achieved a partial response. These cells were administered in conjunction with low dose subcutaneous IL-2. There were no toxicities associated with the cell transfer. Brentjens et al. recently reported on the administration of autologous T cells transduced with a CAR recognizing the CD19 molecule in patients with chronic lymphocytic leukemia. Three patients were treated with modified T cells alone without dose limiting toxicities. However, the first patient who was enrolled in a second cohort of T cell administration following a cyclophosphamide lymphodepleting regimen developed a syndrome of fever and hypotension and died four days later. Following extensive analysis of this patient it

was concluded that the adverse event may have been due to an idiosyncratic reaction to the combination of the cyclophosamide and cell administration. In the Surgery Branch we have an approved clinical protocol to treat patients with B cell lymphomas utilizing their autologous lymphocytes transduced with a retrovirus encoding a CAR that recognizes the CD19 molecule. Thus far two patients has been treated with between 2-4x10^8 cells. The first patient treated has exhibited a near complete response ongoing at over 7 months with elimination of normal B cells. No toxicity was seen. The second patient treated on this study died prior to response evaluation from H1N1 influenza infection and a a fatal cerebral herniation following bilateral cerebral thromboembolism resulting in brain death 15 days after treatment. This death is possibly related to the research but is not related to the anti-CD19 CAR transduced cells.

In a second trial a patient with metastatic colorectal cancer was treated with 10^{10} autologous T cells transduced with the retrovirus encoding an anti-Her-2 CAR. This patient developed respiratory distress and died four days later. This toxicity was apparently due to a previously unrecognized ability of this CAR to recognize Her-2 expressed on lung epithelial cells although the exact explanation for the toxicity is not clear. It is for this reason that in the current protocol we have dropped the dose of CAR transduced cells by 10,000 fold and will start a dose escalation at 10^6 CAR transduced cells. This protocol has been discontinued.

Results of pre-clinical studies conducted in the Surgery Branch in C57BL/6 mice treated with the VEGFR2 CAR demonstrate no toxicity at a dose of 2 x 10^7 DC101-CAR transduced syngeneic T cells. However, in BALB/c mice the transfer of 2 x 10^7 CAR transduced cells was lethal. In BALB/c mice a tumor inhibitory effect was achieved with minimal morbidity if the number of administered T cells was reduced to 5 x 10^6. When CD8 purified CAR transduced cells were administered there was no toxicity seen in BALB/c mice given 2 x 10^7 cells with no diminution of the therapeutic effect. Histopathologic analysis of BALB/c mice treated with 2 x 10^7 DC101-CAR transduced T cells and IL-2 revealed findings characteristic of cytokine induced hypotension including multifocal mild coagulation necrosis in the liver and mild hepatic pericholangitis and pulmonary perivasculitis, villous atrophy, villous blunting, and crypt epithelial hyperplasia of the small intestine and colon. No abnormalities in the gross appearance of the heart, lungs, liver, kidneys, spleen, pancreas, uterus, ovaries, or brain were seen. In the current protocol therefore we will be administering CD8 purified CAR tansduced cells.

Both C57BL/6 and BALB/c mice tolerated 2 x 10^7 CD8 purified CAR transduced cells without toxicity. In the current human protocol the starting cell dose will be 1 x 10^6 cells or 20 fold less cells than tolerated by the mice. Since a 70 Kg human is 3000 fold heavier than a mouse we estimate that our starting cell dose is 60,000 fold lower than that safely tolerated by mice.

Clinical toxicities attributed to VEGFR targeting angiogenesis inhibitors, such as monoclonal antibodies and tyrosine kinase inhibitors, include hypertension, proteinuria, cardiovascular and cerebrovascular thromboembolisms, bleeding, increased rate of gastrointestinal perforations, and hypothyroidism. These toxicities are observed after prolonged administration of the VEGFR targeting angiogenesis inhibitors and are generally easily managed with standard treatments. In the current study, we will administer one dose of the anti-VEGFR2 CAR transduced cells.

In other protocols we have administered over 3 X 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 or uveitis probably due to destruction of normal melanocytes though these toxicities have been manageable. 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.

Prior to amendment C, we accrued 12 patients to this study, with 4 patients accrued to cohort 7 (10^9 cells). Three of the 4 patients accrued to cohort 7 experienced grade 3 or 4 elevated liver enzymes, which are known toxicities of high-dose aldesleukin and are described in the informed consent. After discussions with the FDA, in order to address the possible contribution of aldesleukin to the severity of these events, starting with amendment C the initial dose-escalation schema with high-dose aldesleukin will be stopped and a new dose-escalation schema will start at 10^9 cells (cohort 8), where aldesleukin will be administered intravenously at low doses (72,000 IU/kg every 8h for a maximum of 15 doses).

2.0 Eligibility Assessment and Enrollment

2.1. Eligibility Criteria

2.1.1. Inclusion Criteria

a. Metastatic cancer with evaluable disease.
b. Patients must have previously received at least one systemic standard care (or effective salvage chemotherapy regimens) for metastatic disease, if known to be effective for that disease, and have been either non-responders (progressive disease) or have recurred.
c. Greater than or equal to 18 years of age and less than or equal to 70 years of age.
d. Willing to sign a durable power of attorney
e. Able to understand and sign the Informed Consent Document
f. Clinical performance status of ECOG 0 or 1.
g. Life expectancy of greater than three months.
h. Patients of both genders must be willing to practice birth control from the time of enrollment on this study and for up to four months after treatment.
i. Serology:
    1. 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 be less responsive to the experimental treatment and more susceptible to its toxicities.)

2. Seronegative for hepatitis B antigen, and seronegative for hepatitis C antibody. If hepatitis C antibody test is positive, then patient must be tested for the presence of antigen by RT-PCR and be HCV RNA negative.

j. Hematology:
   1. Absolute neutrophil count greater than 1000/mm^3 without the support of filgrastim.
   2. WBC (> 3000/mm^3).
   3. Platelet count greater than 100,000/mm^3.
   4. Hemoglobin greater than 8.0 g/dl.

k. Chemistry:
   1. Serum ALT/AST less or equal to 2.5 times the upper limit of normal.
   2. Serum creatinine less than or equal to 1.6 mg/dl.
   3. 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.

l. More than four 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).

m. More than 4 weeks must have elapsed since an surgical procedure at the time the patient receives the preparative regimen due to the inhibition of wound healing observed with VEGFR targeting angiogenesis inhibitors.

2.1.2. <u>Exclusion Criteria</u>

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

b. Patients with known brain metastases.

c. Patients receiving full dose anticoagulative therapy.

d. 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.

e. Any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease).

f. 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).

g. Patients with diabetic retinopathy.

h. Concurrent Systemic steroid therapy.

i. History of severe immediate hypersensitivity reaction to any of the agents used in this study.

j. History of coronary revascularization or ischemic symptoms.

k. Documented LVEF of less than or equal to 45% tested in patients with:
   1. History of ischemic heart disease, chest pain, or clinically significant atrial and/or ventricular arrhythmias including but not limited to: atrial fibrillation, ventricular tachycardia, second or third degree heart block.
   2. Age greater than or equal to 60 years old.

2.2. Screening Evaluation

Within **4 weeks** prior to starting the chemotherapy regimen:

a. Complete history and physical examination, including weight and vital signs, and eye exam noting in detail the exact size and location of any lesions that exist. (**Note**: patient history may be obtained within 8 weeks)
b. Chest x-ray
c. EKG
d. Baseline CT of the chest, abdomen and pelvis, 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.
e. Pulmonary Function Testing for patients with a prolonged history of cigarette smoking (20 pk/year of smoking within the past 2 years) or symptoms of respiratory dysfunction (may be performed within 8 weeks of treatment).
f. Evaluation of LVEF in all patients over 60 years old. LVEF will be assessed by either echocardiogram or MUGA scan. Patients under the age of 60 who have cardiac risk factors may also undergo cardiac evaluations as noted above (e.g., diabetes, hypertension, obesity).
g. Patients with a LVEF of less than 45% will not proceed to treatment (may be performed within 8 weeks of treatment).
h. HIV antibody titer and HbsAG determination, anti HCV (may be performed within 3 months of chemotherapy start date).
i. Anti CMV antibody titer, HSV serology, and EBV panel (may be performed within 3 months of chemotherapy start date; patients who are known to be positive for any of the above do not need to be retested).

Within **14 days** prior to starting the chemotherapy regimen:

a. 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)
b. Thyroid panel
c. CBC with differential and platelet count
d. PT/PTT
e. Urinalysis and culture, if indicated

Within **7 days** prior to starting the chemotherapy regimen:

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

2.3. Registration Procedures:

Patients will be registered on protocol 03-C-0277 (Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols) prior to CD8 purification and transduction of CD8+ 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.

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and faxed to 301-480-0757. After confirmation of eligibility at 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 e-mail to the research team. A recorder is available during non-working hours.

3.0 Study Implementation

3.1. Study Design:

3.1.1. Treatment Phase:

3.1.1.1. Cell Preparation

CD8-enriched PBL will be grown, transduced with the anti-VEGFR2 CAR genes and expanded for this trial according to standard operating procedures submitted in the IND. PBMC will be obtained by leukapheresis (approximately 1 X 10^{10} cells). CD8-enriched PBL will be cultured in the presence of anti-CD3 (OKT3) and aldesleukin in order to stimulate T-cell growth. Transduction is initiated by exposure of approximately 1 X 10^{7} to 5 X 10^{8} cells to supernatant containing the anti-VEGFR2 CAR retroviral vector. These transduced cells will be expanded and tested for their anti-tumor activity. Successful CAR gene transfer will be determined by FACS analysis for the CAR protein and anti-VEGFR2 reactivity will be tested by cytokine release as measured on transfected cells. Successful CAR gene transfer for each transduced PBL population will be defined as >30% CAR positive cells and for biological activity, gamma-interferon secretion must be at least 200pg/ml, and greater than twice background.

3.1.1.2. Phase 1 - Dose Escalation: cohorts 1-7 – prior to amendment C (now closed)

Initially, the protocol was designed to evaluate patients who would be treated with a non-myeloablative chemotherapy regimen, VEGFR2 CAR-transduced PBL and high-dose aldesleukin. A total of four patients were treated in cohort 7 and no additional patients will be accrued to this portion of the study. Patients accrued to the new cohorts will receive non-myeloablative chemotherapy, transduced cells and low-dose intravenous aldesleukin. The total number of anti-VEGFR2 CD8+ engineered cells transferred for each cohort will be:

- Cohort 1 10^{6} cells
- Cohort 2 3 X 10^{6} cells
- Cohort 3 10^{7} cells
- Cohort 4 3 X 10^{7} cells
- Cohort 5 10^{8} cells
- Cohort 6 3 X 10^{8} cells
- Cohort 7 10^{9} cells
- Cohort 8 3 X 10^{9} cells
- Cohort 9 10^{10} cells
- Cohort 10 3 X 10^{10} cells

Dosing is based on total cell number rather than % transduced cells since our transduction efficiency with the anti-VEGFR2 CAR genes is between 70-90% in PBL.

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.

In each cohort, if the patient experiences a DLT, five 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, five 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. If there are 1 or fewer DLTs in the first cohort, the study will proceed to the second cohort. If a dose limiting toxicity occurs in the first cohort, that cohort will be expanded to n=6 patients. If two DLTs occur in the first cohort, the study will be terminated.

If interferon-gamma levels post cell infusion increase 5 fold over background and are greater than 100 pg/ml in the patient treated in the current cohort compared to interferon-gamma levels post cell infusion in the patient treated at the prior dose level, the current cohort will be expanded to n=3 to obtain more data on this phenomenon. If one of these 3 patients experiences a DLT, the cohort will be expanded to six patients.

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

Prior to receiving the engineered PBL cells, patients will receive a nonmyeloablative but lymphocyte depleting preparative regimen consisting of cyclophosphamide and fludarabine followed in one to four days by intravenous infusion of in vitro tumor reactive, CAR gene-transduced CD8+ PBL plus IV aldesleukin (720,000 IU/kg q8h for a maximum of 15 doses).

Safety Assessments:

A one week safety assessment period following regimen completion (defined as the last dose of IL-2) will be conducted between each patient in every cohort, except for the last patient as described below.

A two week safety assessment period following regimen completion (defined as the last dose of IL-2) will be conducted after the last patient is treated in each cohort prior to enrollment into the next cohort.

If any unexpected grade 4 or greater toxicity occurs in a patient treated in either the phase 1 or phase 2 portions of this study, a thorough evaluation of the available safety information will be conducted to justify a decision to continue enrolling new subjects into the study.

3.1.1.3. Phase 1 - Dose Escalation (opened with approval of amendment C)

Starting with cohort 8, all subsequent cohorts will proceed in a phase 1 dose-escalation design, with 4 additional cohorts of n=3. However, should 2 DLTs occur in cohort 8, the cell dose will be reduced (cohort 8A). All patient will received non-myeloablative chemotherapy, VEGFR2 CAR-transduced cells and low-dose intravenous aldesleukin. The number of VEGFR2 CAR-transduced cells for each cohort will be:

- Cohort 8 10^9 cells

- Cohort 8A 3×10^8 cells (cell dose de-escalation if 2 DLTs in cohort 8)
- Cohort 9 3×10^9 cells
- Cohort 10 10^{10} cells
- Cohort 11 3×10^{10} cells

Dosing is based on total cell number rather than % transduced cells since our transduction efficiency with the anti-VEGFR2 CAR genes is between 70-90% in PBL.

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.

Following a standard 3+3 design in each cohort, if the patient experiences a DLT, the cohort would be expanded to 6 patients 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, 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 (MTD) prior to starting the phase II portion. If there are 1 or fewer DLTs in the first cohort, the study will proceed to the second cohort. If a dose limiting toxicity occurs in the first cohort of this portion of the study (cohort 8), that cohort will be expanded to n=6 patients. If two DLTs occur in cohort 8, then accrual to cohort 8A (cell dose de-escalation) will start and no more than 1/6 patients may experience a DLT to establish the MTD. If 2 DLTs occur in that cohort, the study will be terminated.

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

Prior to receiving the engineered PBL cells, patients will receive a nonmyeloablative but lymphocyte depleting preparative regimen consisting of cyclophosphamide and fludarabine followed in one to four days by intravenous infusion of in vitro tumor reactive, CAR gene-transduced CD8+ PBL plus IV aldesleukin (72,000 IU/kg q8h for a maximum of 15 doses). 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 3.4.

Safety Assessments:

A one week safety assessment period following regimen completion (defined as the last dose of IL-2) will be conducted between each patient in every cohort, except for the last patient as described below.

A two week safety assessment period following regimen completion (defined as the last dose of IL-2) will be conducted after the last patient is treated in each cohort prior to enrollment into the next cohort.

If any unexpected grade 4 or greater toxicity occurs in a patient treated in either the phase 1 or phase 2 portions of this study, a thorough evaluation of the available safety information will be conducted to justify a decision to continue enrolling new subjects into the study.

3.1.1.4. Phase 2 Portion:

Similar to the Phase 1 portion, prior to receiving the engineered CD8+ PBL cells, patients in the phase 2 portion will receive a nonmyeloablative but lymphocyte depleting preparative regimen consisting of cyclophosphamide and fludarabine followed in one to four days by intravenous infusion of in vitro tumor reactive, CAR gene-transduced CD8+ PBL plus IV aldesleukin (72,000 IU/kg q8h for a maximum of 15 doses). 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 3.4.

The phase 2 portion of the protocol will proceed utilizing the MTD of anti-VEGFR2 CAR CD8+ engineered cells as determined in the phase 1 portion. Patients will be entered into two cohorts based on histology: cohort 1 will include patients with metastatic melanoma and renal cancer, and cohort 2 will include all other cancer types.

See section 3.1.1.2 for the safety assessment to be conducted for any incidence of unexpected grade 4 or greater toxicity.

3.1.2. Definition of Dose Limiting Toxicity (DLT):

Dose-limiting toxicity is defined as follows:

- Grade 2 or greater allergic reaction or reaction that involves bronchospasm or generalized urticaria
- All grade 3 or greater toxicities with the exception of:
    - Grade 3 or 4 myelosuppression, defined as lymphopenia, decreased hemoglobin, neutropenia and thrombocytopenia, due to the chemotherapy preparative regimen that has resolved within 21 days from the day of cell transfer.
    - IL-2 expected toxicities as defined in Appendix 1 and 2.
    - 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.
    - Alopecia, nausea, vomiting, non-hematologic lab abnormalities that persist for less than 96 hours, febrile neutropenia, infection, pain and thrombus related to disease or VAD
    - Expected chemotherapy toxicities as defined in the Pharmaceutical Information section.
    - Grade 3 Fever

3.1.3. Protocol Stopping Rules:

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

- If two DLTs occur in the first cohort of the Phase 1 portion of this study.
- Two or more patients develop a grade 3 or greater toxicity at any point in the study (≥ grade 3 toxicity) not attributable to the chemotherapy preparative regimen or aldesleukin therapy (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.
- If one or more treatment related deaths occur due to the cell infusion, we will promptly discuss this with the NCI IRB and FDA.

3.2. Drug Administration:

(see table 3.2)

3.2.1. Preparative Regimen with Cyclophosphamide and Fludarabine

(*Times are offered as examples and may be changed as long as a similar time relationship between administration of the drugs is maintained. Study medication start times for drugs given once daily should be given within 2 hours of the scheduled time. All other medications should be given +/- one hour of the scheduled time; the length of administration is all +/- 15 minutes. Administration of diuretics, electrolyte replacement, and hydration and monitoring of electrolytes should all be performed as clinically indicated – the doses and times noted below are offered only as examples. Chemotherapy infusions maybe slowed or delayed as medically indicated)*

Days -7 And -6
6 am
Hydrate: Begin hydration with 0.9% Sodium Chloride Injection containing 10 meq/L of potassium chloride at 2.6 ml/kg/hr (starting 11 hours pre-cyclophosphamide and continuing until 24 hours after last cyclophosphamide infusion). At any time during the preparative regimen, if urine output <1.5 ml/kg/hr or if body weight >2 kg over pre-cyclophosphamide value, furosemide 10-20 mg IV maybe administered. Serum potassium should be monitored and treated as indicated following administration of furosemide.

4 pm
Ondansetron (0.15 mg/kg/dose [rounded to the nearest even mg dose between 8 mg and 16 mg based on patient weight] IV every 8 hours X 3 days) will be given for nausea.

5 pm
Cyclophosphamide 60 mg/kg/day X 2 days IV in 250 ml D5W with mesna 15 mg/kg/day X 2 days over 1 hr. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Table 3.

6 pm
Begin mesna infusion at 3 mg/kg/hour intravenously diluted in a suitable diluent (see pharmaceutical section) over 23 hours after each cyclophosphamide dose. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Table 3.

Day -5 to Day -1:
Fludarabine 25 mg/m2/day IVPB daily over 30 minutes for 5 days. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Table 3.

3.2.2. Cell Infusion and Aldesleukin Administration

The anti-VEGFR2 CAR CD8+ PBLs 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.

Day 0 (one to four days after the last dose of the preparative regimen):

- Cells will be infused intravenously (i.v.) on the Patient Care Unit over 20 to 30 minutes (between one and four days after the last dose of fludarabine).
- **With approval of amendment C, low dose aldesleukin will be administered as follows.** Aldesleukin as described in section 3.2.5 below.

Day 1-4 (Day 0 is the day of cell infusion):

- Beginning on day 1 or 2, filgrastim will be administered subcutaneously at a dose of 5 mcg/kg/day (not to exceed 300 mcg/day). Filgrastim administration will continue daily until neutrophil count > 1.0 $x10^9$/L X 3 days or > 5.0 $x10^9$/L.
- **With approval of amendment C, low dose aldesleukin will be administered as follows.** Aldesleukin as described in section 3.2.5 below.

Table 3.2

Day	**-7**	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**1**	**1**	**2**	**3**	**4**
Therapy												
Cyclophosphamide (60 mg/kg)	X	X										
Fludarabine (25 mg/$m^{2)}$			X	X	X	X	X					
Anti-VEGFR2 CAR CD8+ PBL								X[1]				
Aldesleukin								X[2]	X	X	X	X
Filgrastim[3] (5 mcg/kg/day)									X	X	X	X
TMP/SMX[4]	X	X	X	X	X	X	X	X	X	X	X	X
160mg/800mg (example)												
Fluconazole[5] (400 mg po)								X	X	X	X	X
Valacyclovir po or Acyclovir IV[6]								X	X	X	X	X

[1]One to four days after the last dose of preparative regimen
[2]Initiate within 24 hours after cell infusion
[3]Continue until neutrophils count > 1X10^9/L for 3 consecutive days or > 5x10^9/L.
[4]The 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
[5]Continue until ANC > 1000/mm^3
[6]In patients positive for HSV continue until CD4 > 200 X 2

3.2.3. Infection Prophylaxis

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

3.2.3.1. Pneumocystis Jirovecii Pneumonia

All 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) P.O. 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.

3.2.3.2. Herpes Virus Prophylaxis

Patients with positive HSV serology will be given valacyclovir orally at a dose of 500 mg daily the day after chemotherapy ends, or acyclovir, 250 mg/m^2 IV q 12 hrs 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 Pneumocystitis and Herpes will continue for 6 months post chemotherapy. If the CD4 count is less that 200 at 6 months post chemotherapy, prophylaxis will continue until the CD4 count is greater than 200 for 2 consecutive measures.

3.2.3.3. Fungal Prophylaxis

Patients will start Fluconazole 400 mg p.o. the day after chemotherapy concludes and continue until the absolute neutrophil count is greater than 1000/mm^3. 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.

3.2.3.4. Empiric Antibiotics

Patients will start on broad-spectrum antibiotics, either a 3rd or 4th generation cephalosporin or a quinolone for fever of 38.3^{o}C once or two temperatures of 38.0^{o}C or above at least one hour apart, AND an ANC <500/mm^3. Infectious disease consultation will be obtained for all patients with unexplained fever or any infectious complications.

3.2.4. Blood Product Support

Using daily CBC's as a guide, the patient will receive platelets and packed red blood cells (PRBC's) as needed. Attempts will be made to keep Hb >8.0 gm/dl, and plts >10,000/mm3. 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 WBC's and decrease the risk of CMV infection.

3.2.5. Aldesleukin: Intravenous Administration

With approval of amendment C, aldesleukin (based on total body weight) will be administered at a dose of 72,000 IU/kg as an intravenous bolus over a 15 minute

period beginning within 24 hours of the cell infusion and continuing for up to 5 days (maximum 15 doses). Doses will be preferentially administered every eight hours; however, up to 24 hours may elapse between doses depending on patient tolerance. Aldesleukin dosing will be stopped if toxicities are not sufficiently recovered by supportive measures within 24 hours of the last dose of aldesleukin. Doses will be delayed or stopped if patients reach Grade 3 or 4 toxicity due to aldesleukin except for the reversible Grade 3 toxicities common to aldesleukin such as diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes as detailed in Appendix 1. Toxicities will be managed as outlined in Appendix 2. In addition, dosing may be held or stopped at the discretion of the treating investigator (Appendix 3 lists the toxicities seen in patients treated with aldesleukin at the NIH Clinical Center).
Because confusion is a possible side effect of aldesleukin administration, a Durable Power of Attorney will be signed by the patient to identify a surrogate to make decisions if a patient becomes unable to make decisions.

3.3. On-Study Evaluation

Note: Please refer to section 3.3.5 for research evaluations.

3.3.1. Prior to starting the preparative regimen

- Apheresis as indicated (blood specimens may be collected via venipuncture in patients who refuse or are unable to undergo apheresis)
- Within 14 days prior to starting the preparative regimen, patients will have a complete blood count, serum chemistries performed including electrolytes, BUN, creatinine, liver function tests, and TBNK. If any results are beyond the criteria established for eligibility, the patient will not proceed until the abnormalities can be resolved.

3.3.2. During the preparative regimen: DAILY

- Complete Blood Count
- Chem 20: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO^2 (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

3.3.3. After Cell Infusion:

- Vital signs will be monitored hourly (+/- 15 minutes) for four hours and then routinely (every 4 -6 hours) unless otherwise clinically indicated.
- Blood samples will be collected at approximately 1 hour, 4 hours, 24 hours, and 1 week following infusion for analysis of the cytokines IL-6, TNF-α, IFN-γ and GM-CSF.
- Once total lymphocyte count is greater than 200/mm^3, TBNK for peripheral blood CD4 count will be drawn weekly (while the patient is hospitalized). **Please refer to section 3.3.5 for additional post cell infusion evaluations.**

3.3.4. During Hospitalization:

Every 1-2 days

- Review of systems and physical examination as clinically indicated

- CBC
- Chem 20 (Sodium (Na), Potassium (K), Chloride (Cl), Total CO^2 (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.

3.3.5. Additional research evaluations:

3.3.5.1 Prior to Chemotherapy

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

3.3.5.2 Prior to Cell Infusion (one 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.

3.3.5.3 Post cell infusion 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.
- Once total lymphocyte count is greater than 200/mm^3, the following samples will be drawn and sent to the TIL lab on Monday, Wednesday and Friday X 5, then weekly (while the patient is hospitalized):
    - 5 CPT tubes (8 ml each)
    - 1 SST tube (8 ml)
- At other time points, patient peripheral blood lymphocytes (PBL) will be obtained from whole blood by purification using centrifugation on a Ficoll cushion. Aliquots of these PBMC will be 1) cryopreserved for immunological monitoring of cell function and 2) subjected to DNA and RNA extraction for PCR analysis of TCR and vector copy number estimation.

3.3.5.4 Biopsies

Biopsies of tumor tissue or lymph nodes may be performed but are not required during the course of therapy. A maximum of three biopsies will be performed. These biopsies will only be performed if they are superficial and extracavitary. Biopsy tissue will be processed in the Surgery Branch Cell Production Facility in the presence of a Pathology Laboratory pathologist and part of all biopsy tissue will go to the Laboratory of Pathology. Studies will be performed to evaluate the antigen expression by the tumor and to evaluate the reactivity of lymphocytes grown from these biopsies. In addition the presence of transduced cells will be quantitated using RT-PCR for vector sequences.

3.3.5.5 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. Aliquots of these PBMC will be:
    1. Cryopreserved for immunological monitoring of cell function,

2. Subjected to DNA and RNA extraction for PCR analysis of TCR and vector copy number estimation

- Lymphocytes will be tested directly and following in vitro culture. Direct immunological monitoring will consist of quantifying T cells reactive with VEGFR2 by FACS analysis using tetramer staining. Ex vivo immunological assays will consist of cytokine release by bulk PBL (+/- peptide stimulation) and by other experimental studies such as cytolysis 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. Foxp3 levels will be analyzed by semiquantitative RT-PCR to evaluate for mRNA on PBL samples obtained prior to cell infusion and at the follow up time point.
- Blood and tissue specimens collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study if the subject provides permission on the optional studies section of the consent document for protocol 03-C-0277.

3.3.5.6 Monitoring Gene Therapy Trials: Persistence and RCR:

- Engineered cell survival. TCR and vector presence will be quantitated in PBMC samples using established PCR techniques. Immunological monitoring using both tetramer analysis and staining for the TCR will be used to augment PCR-based analysis. This will provide data to estimate the in vivo survival of lymphocytes derived from the infused cells. In addition, measurement of CD4 and CD8 T-cells will be conducted and studies of these T-cell subsets in the circulation will be determined by using specific PCR assays capable of detecting the unique DNA sequence for each retroviral vector engineered T-cell. Note: samples will be batched and assayed at the conclusion of the study.
- All patients will be co-enrolled on protocol 09-C-0161 "follow up Protocol for Subjects Previously Enrolled in NCI Surgery Branch Studies." Patients blood samples will be obtained and undergo analysis for detection of Replication Competent Retroviruses (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. The results of these tests are maintained by the contractor performing the RCR tests and by the Surgery Branch research team.

3.4. Re-Treatment

Patients will be evaluated 4 to 6 weeks after the initial treatment regimen (defined as the end of the last aldesleukin dose). If patients have a partial response to treatment or stable disease that subsequently progresses, they may be re-treated when progression by RECIST criteria is documented after evaluation by principal investigator with the same schedule that they had been given safely (grade III toxicity

due to cell infusion which is reversible within 24 hours with supportive measures may be retreated, but patients who develop grade IV toxicity due to cell infusion will not be retreated.) The duration of a partial response or stable disease to treatment must be greater than or equal to 2 months, as indicated by RECIST criteria, in order to be eligible for retreatment. 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. Cells used for retreatment will consist of cells from the first treatment which have been cryopreserved, if available. If cells from the first treatment are not available, cells for retreatment will consist of a new lot of transduced CD8+ PBL. Toxicity related to cyclophosphamide or fludarabine should be stable and resolved to less than grade 1 prior to retreatment. Re-treatment benefits and risks will be carefully explained to the patient. A maximum of 1 retreatment course may occur. Thirty-six patients have been evaluated after retreatment with a non-myeloablative chemotherapy regimen and autologous cells in the Surgery Branch. Five partial responses and 2 complete responses have been observed for an objective response rate of 19.4%.

3.5. Post Study Evaluation (Follow-up):

3.5.1 All patients will return to the NIH Clinical Center for evaluation 6 weeks (+/- 2 weeks) following the administration of the cell product

3.5.2 Patients discharged with grade 3 or greater significant adverse events should be evaluated by the referring physician within 2 weeks of discharge.

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

- Week 12 (+/- 2 weeks)
- Every 3 months (+/- 1 month) x3
- Every 6 months (+/- 1 month) x 2
- As per PI discretion for subsequent years

Note: Patients may be seen more frequently as clinically indicated

3.5.4 At each evaluation specified above patients will undergo:

- Physical examination
- Toxicity assessment, including a review of systems.
- Lab tests
    - 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, until CD4 > 200 X 2
- CT of the chest, abdomen and pelvis. This end of course evaluation will be used to determine tumor response. 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 pheresis approximately 96 ml of blood may be obtained. Subsequently, approximately 60 ml of blood will be obtained at follow up visits 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: (see section 3.3.6.4)
- 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.

3.5.5 Patients who are unable or unwilling to return for follow up evaluations will be followed via phone or e-mail contacts. Patients may be asked to send laboratory, imaging and physician exam reports performed be their treating physician.

3.6. Criteria for Removal from Protocol Therapy and Off Study Criteria

3.6.1 Off Treatment Criteria:

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

- Completion of the treatment period
- Dose limiting toxicity (DLT) due to cell infusion. The definition of DLT is in Section 3.1.2.
- Grade 3 autoimmunity that involves vital organs (heart, kidneys, brain, eye, liver, colon, adrenal gland, lungs).

3.6.2 Off Study Criteria:

Patients will be taken off study for the following:

- The patient voluntarily withdraws
- There is significant patient noncompliance
- Radiographic or clinical disease progression, unless the patient is eligible for second treatment.
- General or specific changes in the patient's condition render the patient unacceptable for further treatment on this study in the judgment of the investigator.
- Death

Note: patients must be followed until all adverse events have resolved to grade 2 or less with the exception of lymphopenia or alopecia. If an event is not expected to resolve to grade 2 or less, this will be noted in the patient medical record and the patient may be taken off study.

Note: Patients taken off study will be consented and registered on the Surgery Branch follow-up protocol 09-C-0161 (Follow up Protocol for Subjects Previously Enrolled in Surgery Branch Studies)

4.0 Supportive Care

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 q4h), indomethacin (50-75 mg g6h) and ranitidine (150 mg g12h). 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.

5.0 Data Collection and Evaluation

5.1. Response Criteria

5.1.1. Evaluation of target lesions[1]

- Complete Response (CR): Disappearance of all target lesions
- Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions taking as reference the baseline sum LD.
- Progression (PD): At least a 20% increase in the sum of LD of target lesions taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.
- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD.

5.1.2. Evaluation of non-target lesions[2]

- Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level.
- Non-Complete Response: Persistence of one or more non-target lesions
- Progression (PD): Appearance of one or more new lesions. Unequivocal progression of existing non-target lesions

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[1] All measurable lesions up to a maximum of 10 lesions representative of all involved organs should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repetitive measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

[2] All other lesions (or sites of disease) should be identified as **non-target lesions** and should also be recorded at baseline. Measurements are not required, and these lesions should be followed as "present" or "absent."

5.1.3. Evaluation of best overall response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

5.1.4. Confirmatory Measurement/Duration of Response

Confirmation

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat studies that should be performed at least 4 weeks after the criteria for response are first met. In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of 6-8 weeks.

Duration of Overall Response

The duration of overall response is measured from the time measurement criteria are met for CR/PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The duration of overall complete response is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

Duration of Stable Disease

Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

5.2. 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).

Over 100 patients have been treated in the Surgery Branch, NCI with tumor infiltrating lymphocytes. Early toxicities related specifically to the infusion of the cells (those which are seen immediately following cell infusion and prior to aldesleukin administration) are generally mild and include fevers, chills, headache, and malaise. Toxicities which occur following administration of aldesleukin but are thought to be related to the cells include immune mediated events such as vitiligo, transient uveitis, hearing loss and vestibular dysfunction. The use of the non-myeloablative regimen prior to cell administration increases the toxicity of this

treatment as profound myelosuppression occurs in all patients. In 93 patients treated with TIL using the non-myeloablative chemotherapy regimen with or without total body irradiation, there was one treatment related death (NMA + 200 cGY TBI) due to an unexpected but preexisting diverticular abscess.

The standard approach to the administration of high-dose aldesleukin in all studies is to continue dosing until grade 3 or 4 events occur. The most commonly seen grade 4 events are pulmonary, and renal impairment, and mental status changes. These toxicities may sometimes require intubation for protection of the patient's airway. It is important to note that although these patients require significant supportive measures during this period, all toxicities are reversible and the overwhelming majority of patients have suffered no long term sequelae following this treatment regimen. However, fatal complications are possible and it is therefore only appropriate to carry out this experimental treatment in the context of life threatening metastatic cancer.

To ensure safety using this treatment, the NCI SB will review safety data on all protocols semi-annually at the time of continuing review. Data will be presented for both the recent 6 month 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,
- all grade 2 unexpected adverse events, and all grade 3 or greater events regardless of attribution.

Toxicities seen on protocols using this non-myeloablative regimen and aldesleukin, that occur during the follow up period are rare but have included EBV lymphoma following prolonged lymphopenia, herpes zoster infection, and sensory neuropathy likely related to fludarabine.

The major discomforts of the research are those of nausea, mucositis, anorexia, diarrhea, fever and malaise. Side effects of common drugs used in this nonmyeloablative regimen include:

Cyclophosphamide: Marrow suppression, nausea, mucositis, rash, hemorrhagic cystitis, myocardial damage, alopecia, infertility, nausea and vomiting, SIADH.

Fludarabine: Myelosuppression, fever and chills, nausea and vomiting, malaise, fatigue, anorexia, weakness, neurologic toxicity including sensory neuropathies and blindness, and interstitial pneumonitis. Serious opportunistic infections have occurred in CLL patients treated with fludarabine.

Antimicrobials in general: Allergic reactions, renal impairment, nausea, vomiting, hepatic damage, marrow suppression.

Aldesleukin: A variety of side effects have been associated with high-dose aldesleukin administration in our experience and a listing of these side effects in 652

patients who received 1,039 treatment courses are listed in Appendix 1 and Appendix 2. The side effects of low-dose IL-2 have been elucidated from treatment of 150 patients in a Surgery Branch protocol (91-C-0094/T91-0053) from 1991-2001. The most common side effects include nausea/vomiting, oliguria, malaise, changes in level of consciousness, diarrhea, hypotension, peripheral edema, elevated creatinine, and infection

Clinical toxicities attributed to VEGFR targeting angiogenesis inhibitors, such as monoclonal antibodies and tyrosine kinase inhibitors, include hypertension, proteinuria, cardiovascular and cerebrovascular thromboembolisms, bleeding, increased rate of gastrointestinal perforations, and hypothyroidism. These toxicities are observed after prolonged administration of the VEGFR targeting angiogenesis inhibitors and are generally easily managed with standard treatments.

DLT is defined in section 3.1.2.
For safety purposes, the following early stopping rules will apply to this trial:

- If EBV lymphoma in another EBV negative subject occurs in this or any other SB adoptive cell therapy study, accrual of EBV negative subjects to all such studies must halt pending review of the event by the IRB and FDA.
- For additional stopping rules, see Section 3.1.3.

5.3. Statistical Considerations

The primary objectives of this trial is to determine safety and also to determine whether the combination of high-dose aldesleukin, lymphocyte-depleting chemotherapy, and an infusion of anti-VEGFR2 CAR-gene CD8+ engineered lymphocytes is able to be associated with a modest fraction of patients that can experience a clinical response (PR +CR) to therapy. The duration of the clinical response will be followed to assess the efficacy of the regimen. A secondary objective is to have sufficient patients in order to do exploratory evaluations of survival of cells.

Initially, prior to amendment C, the protocol will enroll 1 patient in each of the first 2 dose cohorts unless that patient experiences a dose limiting toxicity (DLT).
Following cohort 2, all subsequent cohorts will proceed in a phase 1 dose escalation design, with 8 cohorts of n=3. The total number of anti-VEGFR2 CD8+ engineered cells transferred for each cohort will be:

- Cohort 1 10^6 cells
- Cohort 2 3×10^6 cells
- Cohort 3 10^7 cells
- Cohort 4 3×10^7 cells
- Cohort 5 10^8 cells
- Cohort 6 3×10^8 cells
- Cohort 7 10^9 cells
- Cohort 8 3×10^9 cells
- Cohort 9 10^{10} cells
- Cohort 10 3×10^{10} cells

In each cohort, if the patient experiences a DLT, five 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, five 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. If a dose limiting toxicity occurs in the first cohort, that cohort will be expanded to n=6 patients. If two DLTs occur in the first cohort, the study will be terminated. If there are 1 or fewer DLTs in the first cohort, the study will proceed to the second cohort.

If interferon-gamma levels post cell infusion increase 5 fold over background and are greater than 100 pg/ml in the patient treated in the current cohort compared to interferon-gamma levels post cell infusion in the patient treated at the prior dose level, the current cohort will be expanded to n=3 to obtain more data on this phenomenon. If one of these 3 patients experience a DLT, the cohort will be expanded to six patients.

Following amendment C, patients will be enrolled onto cohorts 8-11, incorporating low dose aldesleukin and cells, as shown in 3.1.1.3. Since a standard 3+3 design will be used for these cohorts, up to 24 patients may be enrolled in this portion of the trial following the amendment approval.

Once the MTD has been determined, the study then would proceed to the phase II portion. Patients will be enrolled into individual strata depending on their specific histology. This stratification is being used to separate patients who have melanoma or renal cancer, and patients with all other histologies.

For each of the two strata, the phase II portion of the study will be conducted using a phase II optimal design (Simon R, Controlled Clinical Trials 10:1-10, 1989). For the two strata, the objective will be to determine if the combination of high dose aldesleukin, lymphocyte depleting chemotherapy, and anti-VEGFR2 CAR-gene engineered lymphocytes is able to be associated with a clinical response rate that can rule out 5% (p0=0.05) in favor of a modest 20% PR + CR rate (p1=0.20).

In patients in each of the two strata, the following design will be used. For each strata, with alpha=0.05 (5% probability of accepting a poor therapy) and beta=0.10 (10% probability of rejecting a good therapy,), initially 21 evaluable patients will be enrolled. If 0 or 1 of the 21 patients experiences a clinical response, then no further patients will be enrolled. If 2 or more of the first 21 evaluable patients enrolled have a clinical response, then accrual will continue until a total of 41 evaluable patients have been enrolled. As it may take several weeks to determine if a patient has experienced a clinical response, a temporary pause of up to 6 months in the accrual to the trial may be necessary to ensure that enrollment to the second stage is warranted. If 2 to 4 of the 41 have a clinical response, then this will be considered inadequate for further investigation. If 5 or more of 41 patients have a clinical response, then this will indicate that this strategy provides a new approach that may be worthy of further consideration. Under the null hypothesis (5% response rate), the probability of early termination is 72%.

Further, to help ensure that maldistribution of patients who are either particularly responsive or unresponsive in the first stage does not materially interfere with the intended use of the two-stage design, we will aim to enroll 4-5 patients of each allowed histology among the first 21 enrolled in the 'other histology' arm. Although this has its own inherent issues due to limited sample size, since we believe that these 'other histologies' will behave the same clinically, it will permit us to evaluate the different response rates in a limited number of subjects and determine if they differ markedly or not. Since this would merely be an exploratory analysis, we will also look at minor response as well to help evaluate for hints of efficacy. If the response rates do seem to potentially differ markedly by histology, despite our hypothesis that this will not happen, we may consider amending the protocol when appropriate to try to restrict enrollment to those histologies with stronger evidence of responsiveness. For patients with chemotherapy-sensitive tumors (i.e. sarcoma), only responses seen at day 28 and maintained at 4 months will be considered a positive response for accrual to the second phase of this study.

To complete the phase 1 dose-escalation stage, and both cohorts in the phase 2 portion of the study, a total of up to 118 patients may be required (12 patients enrolled up through dose level 7, and up to 24 additional patients in 4 possible escalations for cohorts 8-11 after amendment C in the phase 1 portion for a maximumin of 36 patients in phase 1, and 2 phase 2 cohorts with a maximum of 41 apiece). Provided that about 1-2 patients per month will be able to be enrolled onto this trial, approximately 6 years may be needed to accrual the maximum number of required patients.

5.4. Data and Safety Monitoring Plan

Careful evaluation to ascertain the toxicity, immunologic effects and anti-tumor 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 3.3.7.

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. The NCI IRB will review submitted adverse events monthly to also evaluate trends and will require a follow up plan from the principal investigator whenever a trend is identified.

5.5. 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.

5.6. Sponsor Monitoring Plan

A detailed description of the clinical trial monitoring plan has been included in the initial IND submission as required.

This trial will be monitored by personnel employed by Harris Technical Services on contract to the NCI, NIH. 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.

At least 25% of enrolled patients' will be randomly selected and monitored at least quarterly, base on accrual rate. The patients selected will have 100% source document verification done. Additional monitoring activities will include: adherence to protocol specified study eligibility, treatment plans, data collection for safety and efficacy, reporting and time frames of adverse events to the NCI IRB and FDA, and informed consent requirements. Written reports will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.
Note: The collection and analysis of research labs will be monitored by the TIL lab and not by Harris.

5.7. 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 NCI-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.

5.8. Handling of Tissue Specimens for Research Purposes

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. 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.

Subjects will be given the option to store biospecimens on this protocol, those specimens for which permission for permanent storage and future use has not been granted will be destroyed. 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 NCI-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.

6.0 Human Subjects Protections

6.1. Rationale for Patient Selection

The patients to be entered in this protocol have metastatic cancer which is 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.

6.2. Participation of Children

The use of the nonmyeloablative 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 metastatic cancer, 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.

6.3. Evaluation of Benefits and Risks

The experimental treatment has a chance to provide clinical benefit though this is unknown. The risks in this treatment are detailed in section 5.2. 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.

Aldesleukin is an approved and effective treatment in some patients with metastatic melanoma and renal cancer. In this study, aldesleukin is administered after cell infusion to promote growth and survival of the infused cells. Although aldesleukin can cause serious adverse events, it's use in this protocol is essential to keep the cells alive. The success of this effort cannot be predicted at this time. Because all patients in this protocol have metastatic cancer and limited life expectancies the potential benefit is thought to outweigh the potential risks.

6.4. Consent Document

Patients initially signs a consent when they agree to have PBMC obtained for study and growth either on 03-C-0277, Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols. 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 research nurse, principal investigator, associate investigator, or clinical associate is responsible for obtaining written consent from the patient.

7.0 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 Definitions

7.1.1 Adverse Event

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of at least possibly related to the agent/intervention should be recorded and reported as per sections 7.2, 7.3, 7.5.

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 Problems

Any incident, experience, or outcome that:

- 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**

- Is related or possibly related to participation in the research; **AND**

- Suggests that the research places subjects or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized

7.2 <u>Routine Data Reporting:</u>

All information surrounding patient screening, diagnosis, tumor harvest, treatment, and follow up will be recorded in the patient medical record. Data will be reported via the NCI C3D reporting system. The investigators will be responsible for the collection, maintenance, quality control of the study data.

7.2.1 Routine Adverse Event Reporting

Following registration through 30 days after cell infusion, adverse events will be recorded in the source documents, reviewed by the designated data manager or research nurse or principle investigator and will be captured in C3D. All events occurring during the treatment phase of the study will be followed until resolution to grade 2 or baseline. 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.

7.2.2 Exclusions to Routine Adverse Event Reporting:

Patients will be receiving multiple agents which include commercially available agents (fludarabine, cyclophosphamide and supportive medications) in combination with the investigational agents. Therefore, Grade 2 adverse events 'unrelated' or 'unlikely related' to the investigational agent, and 'possibly', 'probably' or 'definitely' related to the commercially available agents as specified in the package inserts do not require reporting/recording.
During the Phase 2 component of the trial, all grade 1 and 2 events and all grade 2 events unrelated to the cell product events will not be reported/recorded.

7.2.3 Reporting of laboratory events

Laboratory results will be uploaded in C3D however only those grade 3 and 4 eventsthat support the diagnosis of a reportable adverse event or that reflect major organ function will be considered adverse events. For example grade 3 and 4, creatinine, liver function tests, hemoglobin, ANC, ALC, platelets, and lipase and amylase as indicated will be captured as adverse events; electrolytes, BUN, albumin, total protein, uric acid etc, and the remainder of the CBC differential will not be captured as adverse events. For hematological toxicities, the event will not be considered resolved until it reaches grade 2 without the support of transfusions or growth factors.

7.2.4 Reporting of non-laboratory events

For reportable expected adverse events: the adverse event start date will be the date the event reaches a grade 3; the event will be considered resolved once it reaches grade 2.

The highest grade the event reaches in that period will be considered the grade of the event.
For unexpected adverse events, the adverse event start date will be the date the event reaches a grade 2; the event will be considered resolved once it reaches grade 1 or baseline. The highest grade the event reaches in that period will be considered the grade of the event.

7.2.5 Reporting Infections

- Febrile neutropenia will be captured as follows: The start date will be the date the fever of 38.5 or greater was first recorded. The end date will be the date the patient has been afebrile greater than 48 hours or the date the patient develops a clinically significant infection.
- If a patient has a positive culture during the period of febrile neutropenia, the event will be captured as "infection with neutropenia" with the start date as the date the fever of 38.5 was first recorded.
- Infection will only be captured once in any given period regardless of the number of organisms cultured or sites involved.
- Positive cultures seen on routine surveillance cultures with no clinical symptoms will not be captured as infections regardless of whether anti infective agents are given.

7.3 NCI-IRB Reporting

7.3.1 NCI-IRB Expedited Reporting of Adverse Events, Unanticipated Problems, and Deaths

The Protocol PI will report to the NCI-IRB:

- All deaths, except deaths due to progressive disease.
- All Protocol Deviations
- All Unanticipated Problems
- All serious non-compliance

Reports must be received by the NCI-IRB within 7 working days of PI awareness via iRIS.

7.3.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The PI will provide a summary report of adverse events that have occurred on the protocol since the previous continuing review and in aggregate, and interpreted in relation to the risk:benefit of study participants in the narrative.

The protocol PI will report to the NCI-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.

7.3.3 NCI-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 NCI IRB.

7.4 NIH Office of Biotechnology Activities (OBA)/Institutional Biosafety Committee (IBC) Reporting Criteria

7.4.1 Serious Adverse Event Reports to OBA/IBC

The Principal Investigator will notify OBA/IBC via email of any unexpected fatal or life-threatening experience associated with the use of anti-VEGFR2 CAR 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-VEGFR2 CAR PBL, but are not fatal or life-threatening, must be reported to NIH OBA/IBC as soon as possible, but not later than 15 calendar days after the sponsor's initial receipt of the information.

7.4.2 Annual Reports to OBA/IBC

The study Principal Investigator will submit a brief report annually of the progress of the trial within 60 days of the anniversary date that the IND went into effect unless the IND sponsor has been authorized to submit this report.

7.5 FDA Reporting Criteria:

7.5.1 IND Safety Reports to the FDA (Refer to 21 CFR 312.32)

7.5.1.1 Expedited reporting to the FDA

The Sponsor will notify FDA via phone, fax, or email of any unexpected fatal or life-threatening suspected adverse reactions as soon as possible but no later than 7 calendar days of initial receipt of the information using the MedWatch Form 3500a.

The Sponsor is also responsible for reporting any:

- any suspected adverse reaction that is both serious and unexpected
- any findings from clinical, epidemiological, or pooled analysis of multiple studies or any findings from animal or in vitro testing that suggest a significant risk in humans exposed to the drug
- clinically important increase in the rate of a serious suspected adverse reaction over that listed in the protocol or investigator brochure.

to the FDA and to all investigators no later than 15 calendar days after determining that the information qualifies for reporting using the MedWatch Form 3500a. If FDA requests any additional data or information, the sponsor must submit it to the FDA as soon as possible, but no later than 15 calendars days after receiving the request.

7.5.2 FDA Annual Reports (Refer to 21 CFR 312.33)

A report will be submitted annually to the FDA on the progress of the trial within 60 days of the anniversary date that the IND went into effect as indicated in 21CFR 312.33, and any associated FDA correspondences regarding the IND annual report.

8.0 Pharmaceutical Information

8.1. Aldesleukin (Interleukin-2, Proleukin, Recombinant Human Interleukin 2)

How Supplied: Aldesleukin will be provided by the NIH Clinical Pharmacy Department from commercial sources.

Formulation/Reconstitution: Aldesleukin, is provided as single-use vials containing 22 million IU (white mg) aldesleukin as a st lyophilized cake plus 50 mg mannitol and 0.18 mg sodium dodecyl sulfate, buffered with approximately 0.17 mg monobasic and 0.89 mg dibasic sodium phosphate to a pH of 7.5 (range 7.2 to 7.8). The vial is reconstituted with 1.2 mL of Sterile Water for Injection, USP, and the resultant concentration is 18 million IU/ml or 1.1 mg/mL. Diluent should be directed against the side of the vial to avoid excess foaming. Swirl contents gently until completely dissolved. Do not shake. Since vials contain no preservative, reconstituted solution should be used with 24 hours.

Storage: Intact vials are stored in the refrigerator (2° - 8°C) protected from light. Each vial bears an expiration date.

Dilution/Stability: Reconstituted aldesleukin should be further diluted with 50 mL of 5% Human Serum Albumin (HSA). The HSA should be added to the diluent prior to the addition of aldesleukin. Dilutions of the reconstituted solution over a 1000-fold range (i.e., 1 mg/mL to 1 mcg/mL) are acceptable in either glass bottles or polyvinyl chloride bags. Aldesleukin is chemically stable for 48 hours at refrigerated and room temperatures, 2°– 30°C.

Administration: The final dilution of aldesleukin will be infused over 15 minutes. Aldesleukin will be administered as an inpatient.

Toxicities: Expected toxicities of aldesleukin are listed in the product label and in Appendix 1 and 2. Grade 3 toxicities common to aldesleukin include diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes as detailed in Appendix 1. Additional grade 3 and 4 toxicities seen with aldesleukin are detailed in Appendix 2.

8.2. Fludarabine

Description: (Please refer to package insert for complete product Information) 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, ribnucleotide 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 Table 3.

Toxicities: At doses of 25 mg/m^2/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.

8.3. 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 alkyating 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 Table 3.

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-mercaptoethanesulphonate; 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.

8.4. Mesna (Sodium 2-mercaptoethanesulfonate, Mesnum, Mesnex)

(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 to 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 Table 3. Toxicities include nausea, vomiting and diarrhea.

8.5. 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.

8.6. 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.

8.6.1. 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.

8.7. Herpes Virus Prophylaxis

8.7.1. 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. Valcyclovir 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.

8.7.2. 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.

8.8. 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.

8.9. Anti-VEGFR2 CAR transduced CD8+ PBL

The procedure for expanding the human PBL and the Certificate of Analysis (CoA) 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. The CoA is included in Appendix 4 for this protocol, and in the IND submission for these cells along with the the Standard Operating Procedures for the growth of the PBL, purification of the CD8+ cells and transduction with retroviral supernatant containing the chimeric anti-VEGFR2 CAR genes.

8.9.1. Retroviral Vector Containing the anti-VEGFR2 CAR Gene

The retroviral vector supernatant [PG13-KDR1121-hCD828BBZ (D11)] encoding a chimeric antigen receptor (CAR) directed against VEGFR2 was prepared and preserved following cGMP in the Surgery BranchVector Production Facility (SBVPF). The retroviral vector utilizes the MSGV retroviral vector backbone and consists of 7199 bps including the 5' LTR from the murine stem cell virus (promoter), packaging signal including the splicing donor (SD) and splicing acceptor (SA) sites, KDR1121 (anti-KDR)-based CAR protein (KDR1121-CD828BBZ) containing a signal peptide (mouse immunoglobulin kappa chain), KDR1121 light chain variable region (KDR1121 VL), linker peptide, KDR1121 heavy chain (KDR1121 VH), CD8 (hinge and transmembrane regions), CD28 (cytoplasmic

region), 4-1BB (cytoplasmic region), and TCR zeta (cytoplasmic region), followed by the murine stem cell virus 3'LTR. The physical titer will be determined by retroviral transduction of human peripheral blood lymphocytes followed CAR detection by FACS 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 *ex vivo* PBL transduction. 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://bmbl.od.nih.gov/sect3bsl2.htm.

8.10. Support Medications

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.

Furosemide

Furosemide will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to enhance urine output during the chemotherapy preparative regimen with cyclophosphamide. Adverse effects include dizziness, vertigo, paresthesias, weakness, orthostatic hypotension, photosensitivity, rash and pruritis. Consult the package insert for a complete list of all side effects.

9.0 References

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10.0 Tables, Figures, and Appendices:

Table 1: Frequency and duration of objective responses in melanoma patients treated with standard Tumor Infiltrating Lymphocytes (TIL) in the Surgery Branch, NCI (8/1/09)

Treatment*	Total	PR (%)	CR (%)	OR (%)
No TBI#	**43**	**17 (39.5%)** α (84, 52+, 36, 29, 28, 14, 13, 11, 8, 8, 7, 4, 3, 3, 2, 2, 2)	**4 (9.3%)** (80+,77+, 67+, 66+)	**21 (48.8%)**
200cG TBI	**25**	**11 (44.0%)** (52+, 48+, 42+, 14, 10, 6, 5, 5, 4, 3, 3)	**2 (8.0%)** (56+, 45+)	**13 (52.0%)**
1200cG TBI	**25**	**11 (44.0%)** (33+, 26+, 26, 26+, 13, 7, 6, 6, 5, 4, 3)	**7 (28.0%)** (36+, 19, 33+, 32+, 27+, 26+, 25+)	**18 (72%)**

*All patients received cyclophosphamide (60mg/kg x 2d) and fludarabine (25mg/m2 x 5d).

#Patients who received Rapidly Expanded TIL plus the full preparative regimen as a first TIL treatment

∀Durations of response in months are in parenthesis under the number of responders. "+" indicates on ongoing response.
52 responding patients: 42 had prior IL-2, 21 had prior IL-2 + chemotherapy

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%)

[1]Measured from the day of cell administration to discharge

TABLE 3: Modification of Dose Calculations* in patients whose BMI is greater than 35

Unless otherwise specified in this protocol, actual body weight is used for dose calculations of treatment agents. In patients who are determined to be obese (BMI > 35), the **practical weight** (see 3 below) will be used.

1. BMI Determination:

   BMI = weight (kg) / [height (m)]2

2. Calculation of ideal body weight

   Male = 50 kg + 2.3 (number of inches over 60 inches)
   Example: ideal body weight of 5'10'' male
   50 + 2.3 (10) = 73 kg

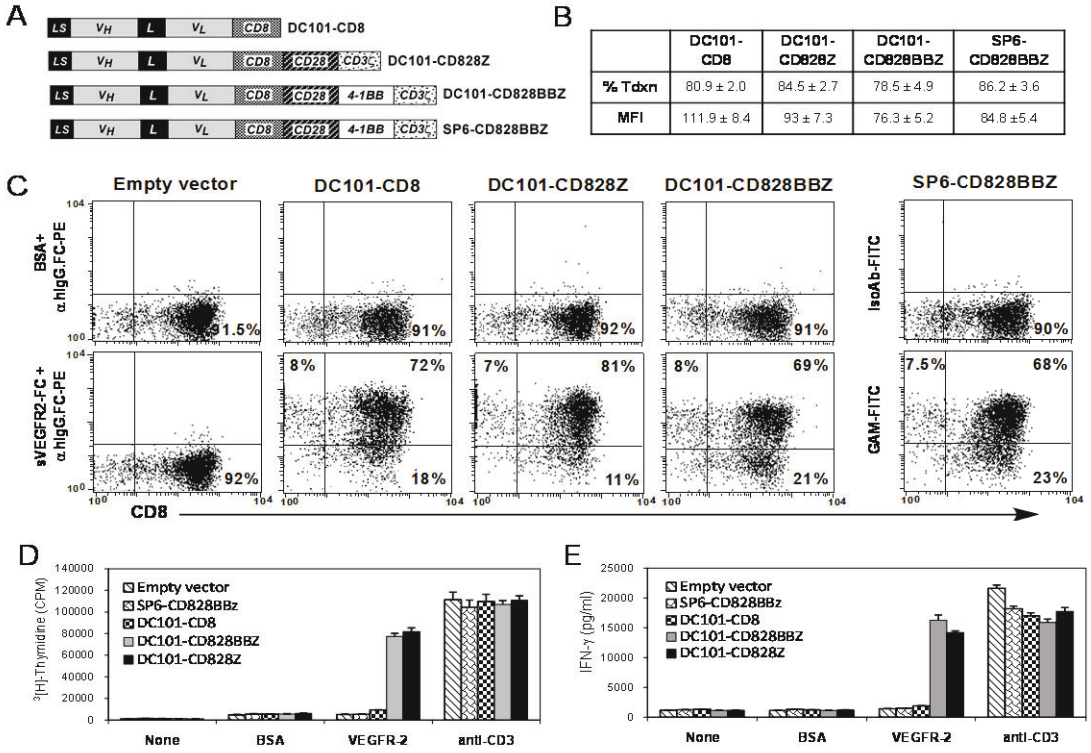
   Female = 45.5 kg + 2.3 (number of inches over 60 inches)
   Example: ideal body weight of 5'3'' female
   45.5 + 2.3 (3) = 57 kg

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.

Figure 1



	DC101-CD8	DC101-CD828Z	DC101-CD828BBZ	SP6-CD828BBZ
% Tdxn	80.9 ± 2.0	84.5 ± 2.7	78.5 ± 4.9	86.2 ± 3.6
MFI	111.9 ± 8.4	93 ± 7.3	76.3 ± 5.2	84.8 ± 5.4

Figure 1: Construction and characterization of recombinant retroviral vectors expressing chimeric antigen receptor (CAR) targeted against VEGFR-2

A) *Schematic representation of recombinant retroviral vector constructs expressing chimeric antigen receptors (CAR) used in this study.* DC101, a single chain antibody (ScFv) comprising the heavy chain (V_H) and light chain (V_L) variable regions of a rat IgG specific to mouse VEGFR-2, (DC101; Imclone Systems Inc.) fused by a 218 bp linker; SP6, ScFv of Sp6 mAb directed against a synthetic hapten TNP SP6. LS, leader sequence; CD8, hinge and transmembrane regions from mouse CD8α; CD28, 4-1BB, and CD3ς, intracellular T cell signaling domains derived from mouse CD28, 4-1BB, and CD3 molecules. DC101-CD8, a binding-control vector encoding DC101 ScFv fused to the hinge and transmenbrane regions from mouse CD8α.

B) *Retroviral mediated expression of various CARs in primary mouse T cells.* Enriched splenic $CD3^+$ T cells were trasduced with various CAR encoding retroviral vectors shown in Figure 1A. On day 4 post transduction, cells were costained with anti-mouse CD8 antibody and CAR-specific reagents and FACS analysed to determine the expression of transgene products in $CD8^+$ and $CD8^-$ T cell subsets. Transduction efficiency and level of expression of CAR in transduced mouse T cells were represented as percent transgene positive cells (% Tdxn) and mean fluorescent intensity of expression (MFI) respectively. Data were represented as mean ± SEM derived from 5 different experiments.

C) *A representative FACS* data of 5 experiments showing the percentage of DC101 CAR or SP6 CAR transduced $CD8^+$ and $CD8^-$ T subsets in the upper right and left quadrants respectively.

D) *Antigen-specific proliferation of DC101 CAR transduced primary mouse T cells in vitro in response to immobilized VEGFR-2 protein.* Microtiter plates were coated with BSA, soluble mouse VEGFR2-hIgG.FC fusion protein, or mouse anti-mouse CD3 mAb as described in material and methods. CD3 enriched mouse T cells were transduced with indicated retroviral vectors as described in material and methods. Four days later cells were cultured on antigen coated micro titer plates for 3 days. At the end of culture period, cells were pulsed with ^{3}[H]-thymidine for 18 hours and analysed for thymidine incorporation as a measure of proliferation. The assays were performed in triplicate and values are represented as mean ± SEM.

E) *DC101 CAR transduced mouse T cells specifically secreted IFN-γ in response to immobilized VEGFR-2 protein.* Transduced mouse T cells were cultured for 2 days on antigen coated microtiter plates as described above. Culture supernatants were harvested and analysed by ELISA for the secretion of IFN-γ. The assays were performed in triplicate and values are represented as mean ± SEM.

Figure 2

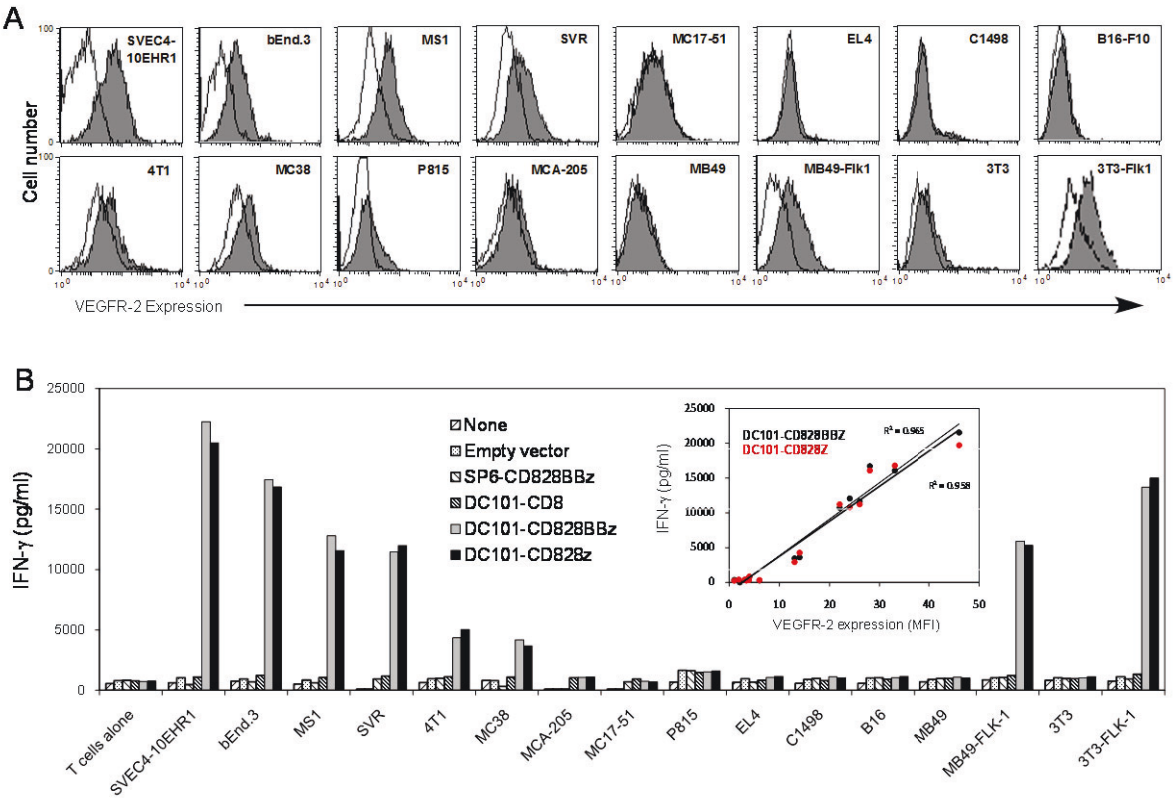


Figure 2: DC101 CAR transduced mouse T cells are functionally competent in generating immune response against VEGFR-2 expressing mouse cell lines.
A) Expression of VEGFR-2 (Flk-1) on mouse endothelial cells and tumor cells. Indicated mouse cell lines were incubated sequentially with recombinant DC101 antibody (a rat anti mouse IgG specific for mouse VEGFR-2) or isotype control antibody (rat IgG1), mVEGFR2-hIgG-Fc fusion protein, and PE-conjugated anti-hIgG-FC and analysed by FACS. Filled histogram VEGFR-2 specific staining; open histogram, staining with isotype control antibody. Results shown are representative of two experiments.
B) Primary mouse T cells transduced with DC101 CAR generated antigen-specific immune response when exposed to VEGFR-2 expressing mouse cell lines. Primary mouse T cells were transduced with various retroviral vector constructs shown in Figure 1A. Four days later cells were cocultured with indicated mouse cell lines. Culture supernatants were harvested at 24 hours post coculture and assayed for IFN-γ by ELISA. Results are presented as the mean values ± SEM of triplicate wells. Correlation between the IFN-γ secretion by DC101 CAR engineered effector T cells and the mean fluorescent intensity of VEGFR-2 expression on the target cells is shown (inset).

Figure 3

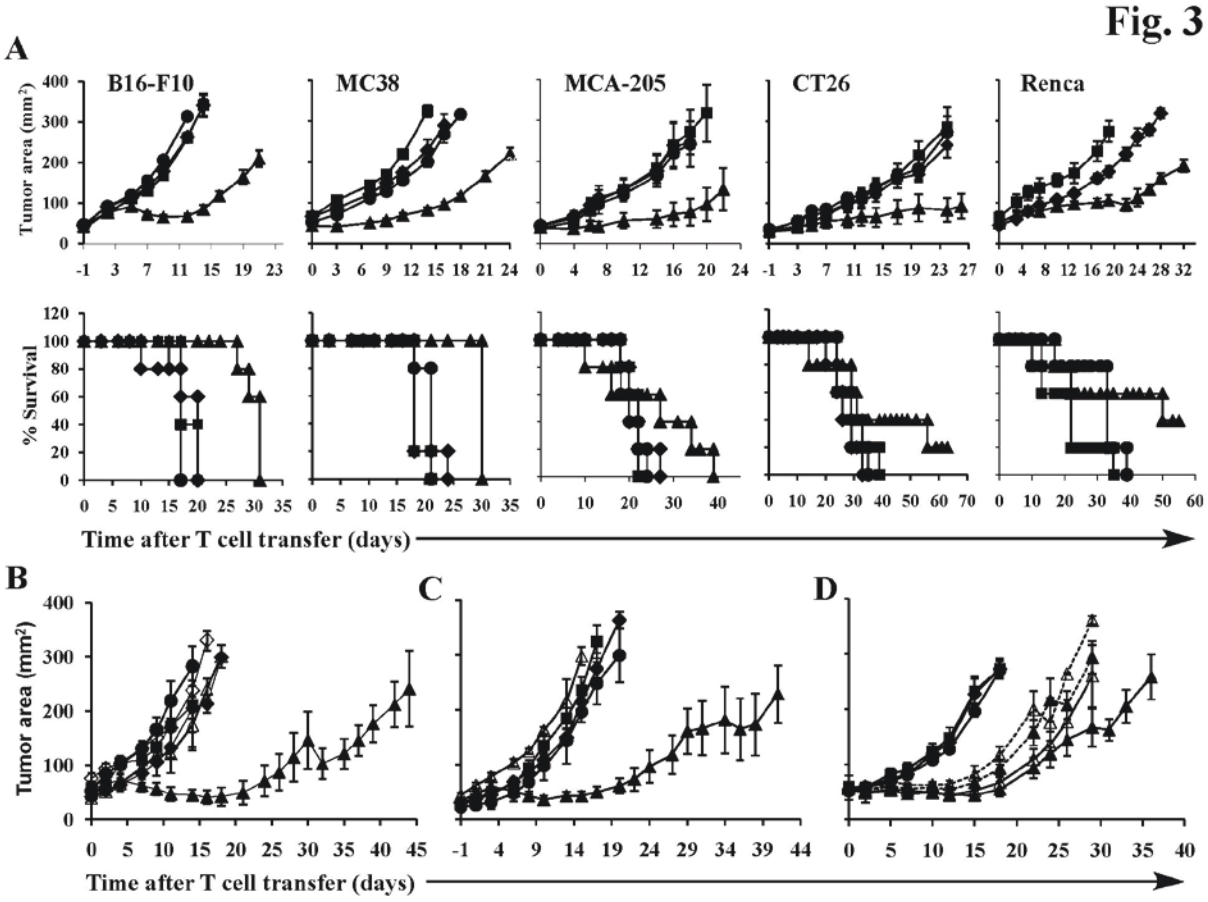


Figure 3: Adoptively transferred DC101-CAR engineered mouse T cells inhibit multiple types of established syngeneic tumors *in vivo*. (**A**) Mice bearing established subcutaneous tumors were sublethally irradiated at 5 Gy TBI and injected with 2 x 10^7 DC101-CAR (closed triangle) or empty vector (closed squares) transduced syngeneic mouse T cells in conjunction with rhIL-2. Control groups received rhIL-2 alone (closed diamond) or no treatment (closed circle). Mice bearing CT26 or Renca tumors were treated with 5 x 10^6 lymphocytes.
(**B**) Anti-tumor effect of DC101-CAR transduced T cells was cell-mediated and not due to an antibody effect. One group in this experiment received a single dose of 800 µg/mouse DC101 antibody (open triangle) or rat IgG1 control antibody (open diamond) plus rhIL-2.
(**C**) C57BL/6 mice bearing subcutaneous B16-F10 tumor were treated with T cells transduced with DC101-CAR (closed triangle) or SP6-CAR vector (closed diamond) containing intact mouse intracellular signaling sequences 28BBZ, DC101-CD8 vector that lacked all the signaling domains (open triangle), or an empty vector (closed square) plus rhIL-2 or were untreated (closed circle).
(**D**) C57BL/6 mice bearing B16-F10 tumor were treated with 2 x 10^7 (solid line with closed triangle), 1 x 10^7 (solid line with open triangle), 5 x 10^6 (dashed line with closed triangle), or 2 x 10^6 (dashed line with open triangle) syngeneic T cells transduced with DC101-CAR (DC101-CD828BBZ) plus rhIL-2. Control groups received 2 x 10^7 T cells transduced with an empty vector plus rhIL-2 (solid square), rhIL-2 alone (solid diamond), or no treatment (solid circle).

Fig. 4

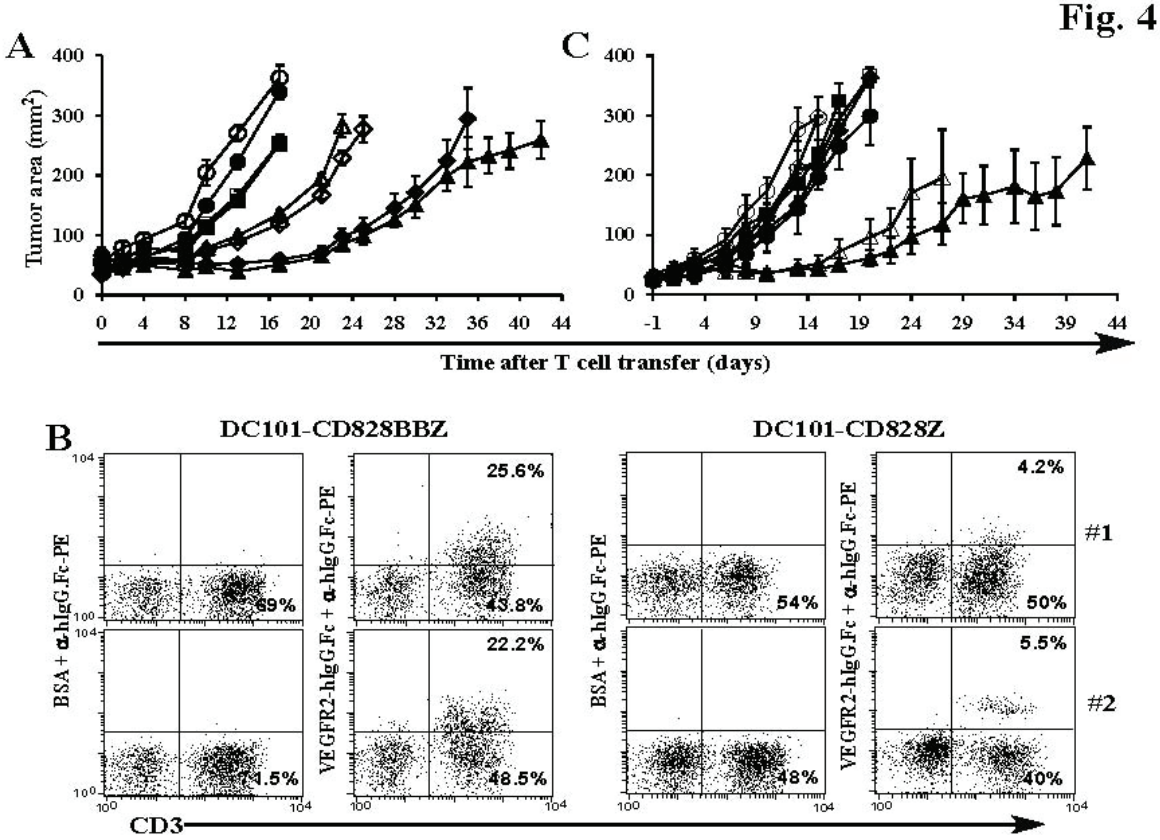


Figure 4: Impact of exogenous rhIL-2, 4-1BB signaling and host lymphodepletion on the tumor treatment effect of DC101 CAR transduced T cells. (**A**) DC101-CAR transduced T cells required exogenous rhIL-2, but not 4-1BB signaling for effective tumor treatment. C57BL/6 mice bearing subcutaneous B16-F10 tumor received 2 x 10^7 syngeneic T cells transduced with DC101-CAR containing the 4-1BB intracellular signaling domain (DC101-CD828BBZ, diamond) or lacking 4-1BB (DC101-CD828Z; triangle) or an empty vector (square). Control groups received no T cell therapy (circle). Groups shown in closed symbols received exogenous rhIL-2 administration and those represented in open symbols did not receive rhIL-2.

(**B**) 4-1BB signaling enhanced the persistence of DC101-CAR modified T cells *in vivo*. Tumor samples from two mice treated with DC101-CAR transduced T cells plus rhIL-2 shown in Fig. 4A were harvested on day 30 post T cell transfer. The low density cell fraction was prepared from each of the tumor samples and the cell surface expression of DC101-CAR was determined by FACS. The percent $CD3^+$ T cells expressing DC101-CAR in the lymphocyte gated region of the forward and side scatter profiles is shown in the upper right quadrants.

(**C**) Impact of host lymphodepletion on *in vivo* tumor therapeutic effect of DC101-CAR engineered T cells. C57BL/6 mice bearing subcutaneous B16-F10 tumors received 2 x 10^7 syngeneic T cells transduced with DC101-CAR (triangle), SP6-CAR (diamond), or an empty vector (square) plus rhIL-2, or not treated with T cells (circle). Mice in groups represented in closed symbols received 5 Gy TBI prior to T cell transfer.

Figure 5

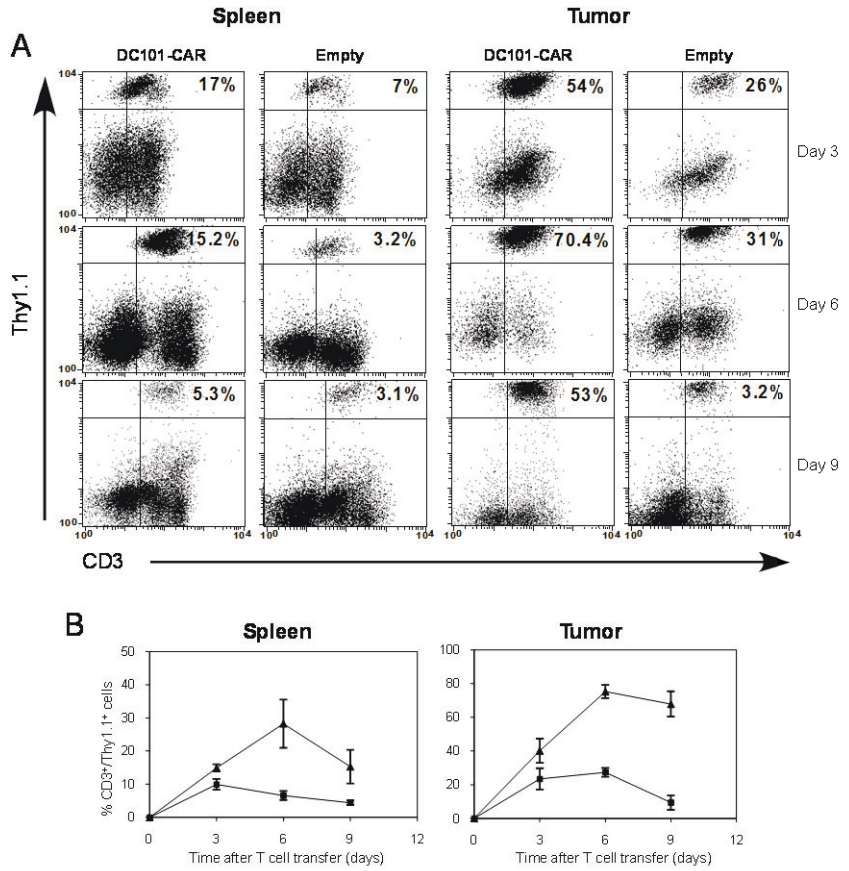


Figure 5: Enhanced tumor infiltration of adoptively transferred DC101 CAR transduced T cells in mice bearing established B16-F10 tumor

C57BL/6 mice were subcutaneously injected with 2×10^5 B16-F10 tumor cells. On day 10 or 12, mice were irradiated at 5 Gy and treated intravenously with 20×10^6 DC101-CD828BBZ CAR or empty vector transduced Thy1.1$^+$ syngeneic T cells in conjunction with rhIL-2. At indicated time points, tumors and spleens from individual mouse from each group were excised and processed to obtain single cell suspensions and analysed by flow cytometry. The percentage of CD3$^+$ Thy1.1$^+$ cells in the lymphocyte gated population of leukocyte fraction of tumor and total splenocytes was determined.

A) A representative FACS data from 3 experiments with similar results.

B) Pooled data obtained from three different mice from independent experiments represented as mean ± SEM.

C-E) Tumor samples were obtained from C57BL/6 mice bearing B16-F10 tumor treated with DC101-CD828BBZ CAR or empty vector transduced T cells in conjunction with rhIL-2 on day 4 post T cell therapy. Tumors sections were stained with FITC conjugated antibody against Thy1.1 antigen expressed on transferred T cells or PE-conjugated antibody against the endothelial cell marker CD31 together with DAPI to show the nucleuos and analysed using fluorescence microscopy.

Figure 6

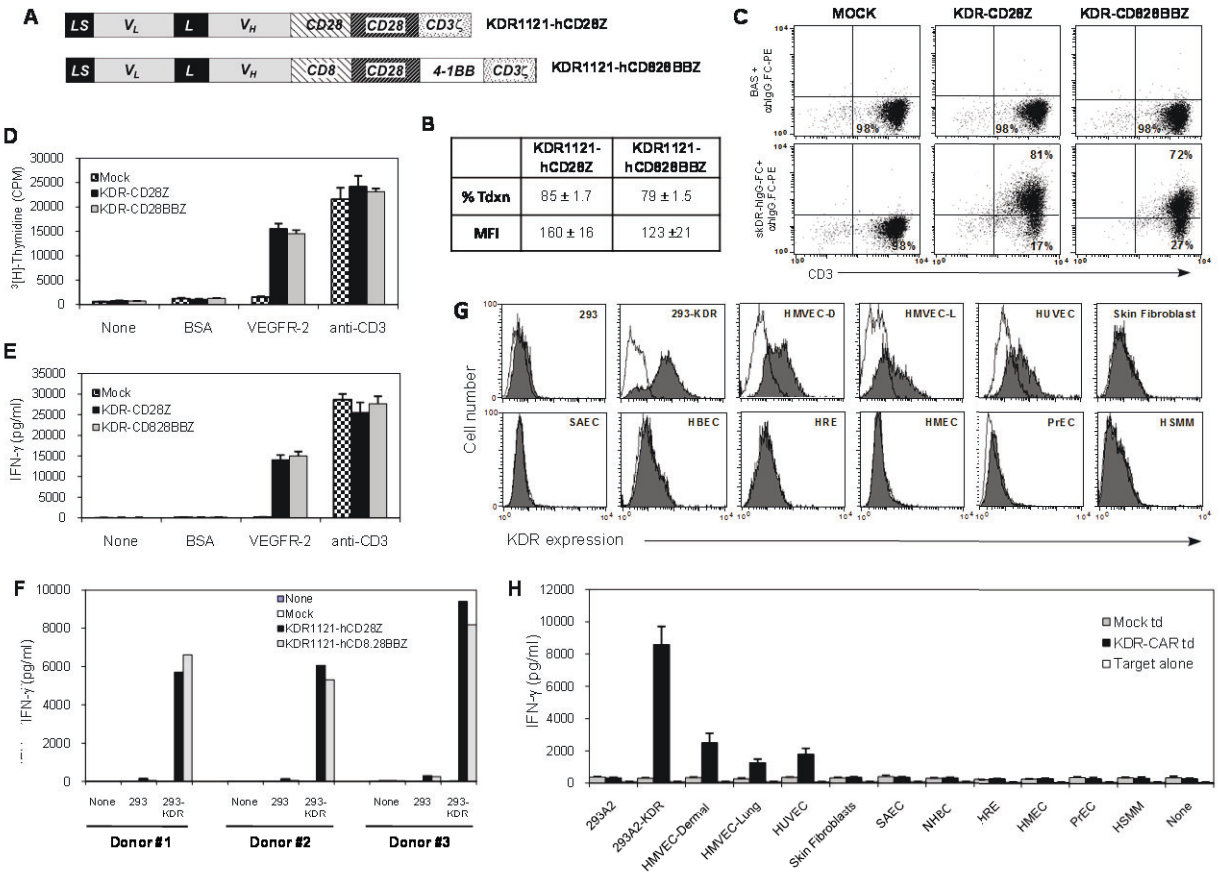


Figure 6: Construction and characterization of retroviral vectors encoding CAR targeted against human VEGFR-2

A) Schematic representation of KDR CAR expressing retroviral vectors. KDR1121, a single chain antibody (ScFv) comprising the variable regions of light chain (VL) and heavy chain (VH) of fully human IgG specific to human KDR antigen (*64*) fused by a 218 bp linker; LS, leader sequence from human GM-CSF; CD8, hinge and transmembrane regions from human CD8α; CD28, 4-1BB, and CD3ς, intracellular T cell signaling domains derived from human CD28, 4-1BB, and CD3 molecules respectively.

B) *Retroviral mediated expression of various CARs in primary human T cells.* OKT3 activated human PBLs were transduced with various CAR encoding retroviral vectors shown in Figure 6A or mock transduced. On day 5 post transduction, cells were costained with anti-human CD3 antibody and KDR CAR-specific reagents and FACS analysed. KDR CAR expression was detected by staining with soluble KDR-hIgG-Fc fusion protein followed by PE-conjugated anti human IgG-FC (αhIgG.FC). Transduction efficiency and level of expression of KDR CAR in transduced primary human T cells are represented as percent transgene positive cells (% Tdxn) and mean flurescent intensity of expression (MFI), respectively. Data are represented as mean ± SEM of data derived from 3 different donors.

C) A representative FACS data from 3 different donors transduced similarly with KDR1121-CD828BBZ or KDR1121-hCD28Z CAR encoding retroviral vectors showing the percentage

of KDR $CAR^{+}CD3^{+}$ T cells and KDR $CAR^{-}CD3^{+}$ T cells in the upper right and lower right quadrants respectively.

D) *Antigen-specific proliferation of KDR CAR transduced primary human T cells in vitro in response to immobilized KDR protein.* Microtiter plates were coated with BSA, soluble KDR-hIgG.FC fusion protein, or anti-human CD3 mAb. OKT3 stimulated human PBLs were transduced with indicated retroviral vectors or mock transduced as described in Figure 6B. Seven days later cells were cultured on antigen coated microtiter plates for 3 days. At the end of culture period, cells were pulsed with ^{3}[H]-thymidine for 18 hours and analysed for thymidine incorporation as a measure of cell proliferation. The assays were performed in triplicate and values are represented as mean ± SEM.

E) *KDR CAR transduced human T cells specifically secreted IFN-γ in response to immobilized KDR protein.* Transduced human PBLs were cultured for 2 days on antigen coated microtiter plates as described above. Culture supernatants were harvested and analysed by ELISA for the secretion of IFN-γ. The assays were performed in triplicate and values are represented as mean ± SEM.

F) *KDR CAR transduced human T cells specifically secreted IFN-γ in response to KDR expressing 293 cells.* Primary human lymphocytes obtained from 3 different donors were transduced with KDR1121-hCD828BBZ or KDR1121-hCD28Z encoding retroviral vectors or mock transduced. Eight days later, 10^{5} transduced T cells were cocultured with 10^{5} indicated human cells in 96 well microtiter plates in 200 μl culture volume/well. Culture supernatants were harvested at 24 hours post coculture and assayed for IFN-γ by ELISA. Results are presented as the mean values of duplicate wells.

G) *KDR expression on various normal primary human cells.* Indicated human cell types were stained with PE- conjugated mouse anti-human KDR antibody or isotyped matched IgG1 control antibody and analysed by FACS. Filled histogram KDR-specific staining; open histogram, staining with isotype control antibody. Results shown are representative of two experiments.

H) *Primary human T cells transduced with KDR CAR generated antigen-specific immune response when exposed to KDR expressing human cells.* Primary human T cells were mock transduced or transduced with KDR1121-hCD828BBZ CAR expressing retroviral vector. Eight days later cells were cocultured with indicated human cells. Culture supernatants were harvested at 24 hours post coculture and assayed for IFN-γ by ELISA. Results are presented as the mean values ± SEM of triplicate wells.

Appendix 1:

ADVERSE EVENTS OCCURRING IN ≥10% OF PATIENTS TREATED WITH ALDESLEUKIN (n=525)[1]

Body System	% Patients
Body as a Whole	
Chills	52
Fever	29
Malaise	27
Asthenia	23
Infection	13
Pain	12
Abdominal pain	11
Abdomen enlarged	10
Cardiovascular	
Hypotension	71
Tachycardia	23
Vasodilation	13
Supraventricular tachycardia	12
Cardiovascular disorder[a]	11
Arrhythmia	10
Digestive	
Diarrhea	67
Vomiting	50
Nausea	35
Stomatitis	22
Anorexia	20
Nausea and vomiting	19
Hemic and Lymphatic	
Thrombocytopenia	37
Anemia	29
Leukopenia	16
Oliguria	63

Body System	% Patients
Metabolic and Nutritional Disorders	
Bilirubinemia	40
Creatinine increase	33
Peripheral edema	28
SGOT increase	23
Weight gain	16
Edema	15
Acidosis	12
Hypomagnesemia	12
Hypocalcemia	11
Alkaline phosphatase incr	10
Nervous	
Confusion	34
Somnolence	22
Anxiety	12
Dizziness	11
Respiratory	
Dyspnea	43
Lung disorder[b]	24
Respiratory disorder[c]	11
Cough increase	11
Rhinitis	10
Skin and Appendages	
Rash	42
Pruritus	24
Exfoliative dermatitis	18
Urogenital	

a Cardiovascular disorder: fluctuations in blood pressure, asymptomatic ECG changes, CHF.
b Lung disorder: physical findings associated with pulmonary congestion, rales, rhonchi.
c Respiratory disorder: ARDS, CXR infiltrates, unspecified pulmonary changes.
[1]Source: Proleukin® Prescribing Information – June 2007

Appendix 2

Expected IL-2 Toxicities and their Management

Expected toxicity	Expected grade	Supportive Measures	Stop Cycle*	Stop Treatment **
Chills	3	IV Meperidine 25-50 mg, IV q1h, prn,	No	No
Fever	3	Acetaminophen 650 mg, po, q4h; Indomethicin 50-75 mg, po, q8h	No	No
Pruritis	3	Hydroxyzine HCL 10-20 mg po q6h, prn; Diphenhydramine HCL25-50 mg, po, q4h, prn	No	No
Nausea/ Vomiting/ Anorexia	3	Ondansetron 10 mg, IV, q8h, prn; Granisetron 0.01 mg/kg IV daily prn; Droperidol 1 mg, IV q4-6h, prn; Prochlorperazine 25 mg pr, prn or 10 mg IV q6h prn	No	No
Diarrhea	3	Loperamide 2mg, po, q3h, prn; Diphenoxylate HCl 2.5 mg and atropine sulfate 25 mcg, po, q3h, prn; codeine sulfate 30-60 mg, po, q4h, prn	If uncontrolled after 24 hours despite all supportive measures	No
Malaise	3 or 4	Bedrest	If other toxicities occur simultaneously	No
Hyperbilirubinemia	3 or 4	Observation	If other toxicities occur simultaneously	No
Anemia	3 or 4	Transfusion with PRBCs	If uncontrolled despite all supportive measures	No
Thrombocytopenia	3 or 4	Transfusion with platelets	If uncontrolled despite all supportive measures	No
Edema/Weight gain	3	Diuretics prn	No	No
Hypotension	3	Fluid resuscitation Vasopressor support	If uncontrolled despite all supportive measures	No

Dyspnea	3 or 4	Oxygen or ventilatory support	If requires ventilatory support	No
Oliguria	3 or 4	Fluid boluses or dopamine at renal doses	If uncontrolled despite all supportive measures	No
Increased creatinine	3 or 4	Observation	Yes (grade 4)	No
Renal failure	3 or 4	Dialysis	Yes	Yes
Pleural effusion	3	Thoracentesis	If uncontrolled despite all supportive measures	No
Bowel perforation	3	Surgical intervention	Yes	Yes
Confusion	3	Observation	Yes	No
Somnolence	3 or 4	Intubation for airway protection	Yes	Yes
Arrhythmia	3	Correction of fluid and electrolyte imbalances; chemical conversion or electrical conversion therapy	If uncontrolled despite all supportive measures	No
Elevated Troponin levels	3 or 4	Observation	Yes	If changes in LV function have not improved to baseline by next dose
Myocardial Infarction	4	Supportive care	Yes	Yes
Elevated transaminases	3 or 4	Observation	For grade 4 without liver metastases	If changes have not improved to baseline by next dose
Hyperbilirubinemia	3 or 4	Observation	For grade 4 without liver metastases	If changes have not improved to baseline by next dose
Electrolyte imbalances	3 or 4	Electrolyte replacement	If uncontrolled despite all supportive measures	No
Neutropenia	4	Observation	No	No

*Unless the toxicity is not reversed within 12 hours

** Unless the toxicity is not reversed to grade 2 or less by next treatment.

Appendix 3: Interleukin-2 toxicities observed in patients treated at the NIH Clinical Center

TABLE 8. *Toxicity of Treatment with Interleukin-2*

Interleukin-2 Plus	Alone	TNF	a-IFN	MoAB	CYT	LAK	TIL	Total
Number of Patients	155	38	128	32	19	214	66	652*
Number of Courses	236	85	210	35	30	348	95	1039
Chills	75	16	68	8	8	191	33	399
Pruritus	53	9	26	2	2	82	6	180
Necrosis	3	—	2	—	—	—	—	5
Anaphylaxis	—	—	—	1	—	—	—	1
Mucositis (requiring liquid diet)	6	1	7	—	2	12	2	30
Alimentation not possible	1	—	1	—	—	2	—	4
Nausea and vomiting	162	42	117	14	20	263	48	666
Diarrhea	144	38	98	15	13	250	38	596
Hyperbilirubinemia (maximum/mg %)								
2.1–6.0	126	49	97	21	18	190	46	547
6.1–10.0	49	3	12	8	9	72	26	179
10.1+	26	1	4	3	1	40	8	83
Oliguria								
<80 ml/8 hours	81	37	67	14	9	114	25	347
<240 ml/24 hours	19		2	3	1	12	5	42
Weight gain (% body weight)								
0.0–5.0	106	23	65	8	9	117	49	377
5.1–10.0	78	41	111	22	10	148	26	436
10.1–15.0	43	17	26	3	9	62	15	175
15.1–20.0	7	3	8	1	1	15	3	38
20.1+	2	1		1	1	6	2	13
Elevated creatinine (maximum/mg %)								
2.1–6.0	148	43	121	20	14	237	54	637
6.1–10.0	21	1	14	3	—	34	12	85
10.1+	5	—	1	1	—	2	1	10
Hematuria (gross)	—	—	—	—	—	2	—	2
Edema (symptomatic nerve or vessel compression)	4	—	6	—	—	7	—	17
Tissue ischemia	—	—	—	—	1	1	—	2
Resp. distress:								
not intubated	17	1	9	4	1	28	7	67
intubated	15	—	6	3	—	12	5	41
Bronchospasm	2	—	2	—	1	4	—	9
Pleural effusion (requiring thoracentesis)	4	1	—	1	2	8	1	17
Somnolence	29	2	22	6	2	45	8	114
Coma	9	1	8	—	2	8	5	33
Disorientation	52	3	50	7	4	89	10	215
Hypotension (requiring pressors)	119	16	40	17	12	259	45	508
Angina	5	1	8	—	—	8	—	22
Myocardial infarction	4	—	1	—	—	1	—	6
Arrythmias	15	2	13	3	—	39	6	78
Anemia requiring transfusion (number units transfused)								
1–15	77	16	53	9	6	176	40	377
6–10	22	1	5	3	2	53	9	95
11–15	4	—	1	—	—	15	4	24
16+	1	—	1	—	—	11	1	14
Thrombocytopenia (minimum/mm^3)								
<20,000	28	1	2	4	6	71	19	131
20,001–60,000	82	11	62	14	12	150	30	361
60,001–100,000	53	36	76	11	8	79	22	285
Central line sepsis	13	—	7	1	4	36	2	63
Death	4	—	1	—	—	3	2	10

* Eleven patients are in two protocols.

Appendix 4

Certificate of Analysis:
Infused T cells transduced with anti-VEGFR2 CAR

Date of preparation of final product:
Patient:

Tests performed on final product:

Test	*Method*	*Limits*	*Result*	*Initials/ Date*
Cell viability[1]	trypan blue exclusion	>70%		
Total viable cell number[1]	visual microscopic count	Between 10^6 and 5×10^{10} cells		
Identity	FACs	> 80 % CD3+CD8+ after transduction on REP cells		
Tumor reactivity[2]	γ-IFN release vs. VEGFR2 cell line	>200 pg/ml and > 2 times background		
CAR expression[2]	FACS analysis of the transduced cells	PBL, >30%		
Microbiological studies	gram stain[1,3,]	no micro-organisms seen		
	aerobic culture[3,4]	no growth		
	fungal culture[3,4]	no growth		
	anaerobic culture[3,4]	no growth		
	mycoplasma test[5]	negative		
Endotoxin	limulus assay[1]	<5 E.U./kg		
RCR	S+L- Assay[4] RCR-PCR[6]	negative		

[1] Performed on sample of the final product immediately prior to infusion. Results are available at the time of infusion.
[2] Performed 2-10 post transduction. Results are available at the time of infusion.
[3] Performed 2-4 days prior to infusion. Results are available at the time of infusion but may not be definitive.
[4] Sample collected from the final product prior to infusion. Results will not be available before cells are infused into the patient.
[5] Performed 2-10 days prior to infusion. Results are available at the time of infusion.
[6] Performed on sample approximately 1-4 days prior to infusion. Results are available at the time of infusion.

Prepared by: _____ Date: _____

QC sign-off: _____ Date: _____
Qualified Clinical or Laboratory Supervisor