Protocol with Statistical Analysis Plan Cover Page:

Official Title:	A Phase I Study of HERV-E TCR Transduced Autologous T Cells in Patients with Metastatic Clear Cell Renal Cell Carcinoma
NCT number:	NCT03354390
Document Type:	Protocol with Statistical Analysis Plan (SAP)
Document Date:	May 11, 2023

CLINICAL RESEARCH PROJECT

Protocol #18-H-0012
IND #17690

Date: May 11, 2023

Title: A Phase I Study of HERV-E TCR Transduced Autologous T Cells in Patients with Metastatic Clear Cell Renal Cell Carcinoma

Other identifying words: kidney cancer, genetically modified lymphocytes, tumor antigens, T cell receptor immunotherapy, genomic retroviral elements.

Principal Investigator:
Richard W. Childs, M.D.,
Cellular and Molecular Therapeutics Branch (CMTB)
National Heart Lung and Blood Institute
Building 10, Room CRC 3-5330
9000 Rockville Pike,
Bethesda, MD 20892
Phone: 301-451-7128
Email: childsr@nhlbi.nih.gov

Loyola University Site Investigators:
Michael I. Nishimura, PhD. Site Principal Investigator
Gina Scurti, PhD.
Annika Dalheim

Subjects:	Number	Sex	Age range
Recipients:	up to 24	Either	18-75

Project involves ionizing radiation?	Yes (medically and research indicated)
Off-site project?	Yes (for cell processing and TCR transduction only)
Multi-institutional project?	Yes
DSMB Involved?	Yes
Tech Transfer?	Yes

Investigational Agent

Drug Name:	HERV-E TCR transduced CD8+/CD34+-enriched T cells
IND Number:	17690
Sponsor:	NHLBI OCD
Manufacturer:	Dr. Michael Nishimura's Laboratory

1. PRECIS

Metastatic renal cell carcinoma (RCC) is an incurable condition. Current therapy for this disease consists of the serial administration of agents such as VEGF, mTOR inhibitors and immunotherapy (high-dose (HD) IL-2 or immune-checkpoint inhibitors). Long-term survival can be achieved with high-doses IL-2 or immune-checkpoint inhibitors. However, of those patients treated with immunotherapy, three quarters will not respond at all and only 5-8% will achieve a complete and durable response.

Allogeneic hematopoietic stem cell transplantation is also capable of inducing prolonged disease regression in patients with metastatic clear cell RCC (ccRCC). In vitro studies have established that transplanted donor T-cells targeting antigens expressed on RCC cells mediate these anti-tumor effects. However, hematopoietic stem cell transplantation can be toxic and associated with a 10-20% risk of procedure-related mortality. The observation that transplanted donor T-cells have the potential to cure a subset of patients with metastatic disease forms the basis for continued efforts in our laboratory to harness the power of T-cells to cure this disorder.

Our team isolated a tumor-specific cytotoxic T lymphocyte (CTL) line from peripheral blood mononuclear cells (PBMCs) obtained after an allogeneic transplant from a patient who showed prolonged tumor regression. Using limiting dilution cloning, we identified an allogeneic (derived from the stem cell donor) CD8+ T-cell clone that killed ccRCC cells in an HLA A11 restricted fashion. Using cDNA expression cloning, we identified a HERV-E derived antigen expressed in the patient's ccRCC cells to be the target of this T-cell clone. Remarkably, we found this HERV-E was expressed in the majority of ccRCC cells with no expression in normal tissues. Based on the identification of the antigenicity of the HERV-E transcripts in ccRCC, our team in collaboration with Dr. Nishimura's laboratory (Loyola University Cardinal Bernardin Cancer Center) has cloned, expressed and characterized the TCR from this CD8+ T-cell clone that recognizes an HLA A11 restricted HERV-E antigen.

This research protocol is therefore designed to evaluate the safety and effectiveness of infusion of HERV-E TCR transduced CD8+/CD34+ enriched T cells in HLA-A*11:01 positive patients with metastatic clear cell RCC. Subjects will receive a novel non-myeloablative immunosuppressive conditioning regimen of cyclophosphamide and fludarabine followed by an infusion of HERV-E TCR transduced CD8+/CD34+ enriched T cells. To mediate T cell survival and sustain function, moderate-doses of IL-2 (aldesleukin) will be administered intravenously twice a day for 14 doses.

The primary endpoint is safety by day 21. Secondary endpoints will include overall response rate, progression-free survival and overall survival. Exploratory studies will include persistence of circulating HERV-E TCR transduced CD8+/CD34+ enriched T cells, changes in immune cell subsets and activation status of T cells, as well as, other immunologic determinants with clinical outcomes at baseline, at different time points during treatment and at the time of disease progression.

TABLE OF CONTENTS

OBJECTIVES
Primary Objectives:

- To determine the safety of infusing escalating doses of HERV-E TCR transduced $CD8^+/CD34^+$ enriched T cells in patients who are HLA-A*11:01 positive who have progressive metastatic clear cell renal cell carcinoma.

Secondary Objectives:

- To assess for tumor response using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 criteria, as well as progression-free survival (PFS) and overall survival (OS).
- To evaluate biologic and immunologic parameters to understand and improve the safety and efficacy of these TCR transduced T cells in metastatic ccRCC patients.

2. BACKGROUND

The worldwide incidence of renal cell carcinoma (RCC) is increasing. Each year >300,000 new diagnoses and more than 140,000 deaths are recorded. RCC is also the most frequent form of kidney cancer accounting for 2.4% of all adult cancers and 90% of all kidney cancers.[1] The most common subtypes are clear cell (cc)RCC, accounting for 70–75% of cases.[2] Sixty to seventy percent of patients present with localized disease (stages I or II), for which radical or partial nephrectomy is largely curative with 5-year survival rates greater than 70%. Patients diagnosed with stage III and IV RCC have poorer prognosis, with 5-year survival rates of approximately 50% and 10%, respectively.[3] Metastatic RCC (mRCC) is resistant to chemotherapy and radiotherapies and is in most cases incurable.

The genetics of clear cell RCC
A modest proportion (2–4%) of RCC is associated with the von Hippel–Lindau (VHL) syndrome. This familial form of RCC is caused by germ line mutations in the VHL tumors suppressor gene on chromosome 3p.25.[4] Pioneering investigations of VHL in sporadic ccRCC, the most common form of RCC, showed that VHL is inactivated by mutation or epigenetic changes in >90% of ccRCC, establishing this gene as a major driver of ccRCC. [5-7] Inactivating genetic alterations in VHL leads to constitutive stabilization of HIF-α and the formation of a HIF-α–HIF-β complex. This complex translocates to the nucleus and stimulates the expression of HIF target genes, which are involved in angiogenesis, glycolysis, cell proliferation, invasion and metastasis.[3]

Genomic studies have also revealed that the PI3K–mTOR pathway, which has a regulatory role in hypoxia signaling and affects the activity of HIF-1α, is widely disrupted in ccRCC.[8] In fact, the functional relevance of aberrant mTOR signaling was first demonstrated by the observation that deficiency of PTEN,[9,10] and activation of AKT,[11] were associated with poor prognosis. Subsequently, 27–28% of ccRCC tumors were found to be affected by genetic alterations in the PI3K–mTOR pathway.[12,13]

Overall, 80% of ccRCC tumors harbor non-synonymous mutations in epigenetic regulatory pathways, emphasizing the important role of epigenome aberrations in this disease. These genes include the SWI/SNF chromatin remodeling gene PBRM1(mutated in 40% of patients); SETD2, (mutated in 19% of patients); and the BRCA1-interacting deubiquitinase gene BAP1 (mutated in 12% of patients).[12,14,15] These three genes are near VHL on chromosome 3p and are affected by LOH in 90% of patients. Other epigenetic modifiers also harbor somatic mutations in ccRCC, including the X-linked genes KDM5C (also known as JARID1C)[16] and KDM6A (also known as UTX).[17]

Furthermore, increased levels of tumor cell dedifferentiation and other sarcomatoid features are present in 1–8% of RCC tumours,[18] of which 65% exhibit clear cell histology.[19] Patients with sarcomatoid ccRCC have a much worse prognosis than those with non-sarcomatoid tumours;[20] however, little is known about the etiology of these tumors and the mechanisms underlying their aggressive behavior.[21] Although sarcomatoid and carcinomatous components within the same tumors harbor equivalent mutations in genes known to drive ccRCC, the available sequence data suggest that sarcomatoid elements also harbor unique mutations in other cancer- related genes such mutations in *TP53*, and have a higher overall somatic mutation rate.[22,23]

Treatment of clear cell RCC

The treatment of RCC has been transformed by recognition of its immunogenic properties and pathogenesis (described previous paragraph). Prior to 2005, treatment options for mRCC were limited, but evidence existed that this tumor is particularly sensitive to manipulations of the immune system. Everson and Cole were among the first to describe spontaneous remissions in patients with mRCC when they reported cases of tumors exhibiting shrinkage without any substantial treatment in the 1960s.[24,25] IL-2, a cytokine important for regulating circulating lymphocytes, was found to result in durable complete remissions in about 5–7% of clear cell mRCC patients, leading to the approval of aldesleukin by the US FDA for this population in 1992. Unfortunately, most patients did not respond and this treatment was extremely toxic, limiting its use to experienced, high volume centers and reserved for patients with a good performance status and acceptable comorbid conditions.[26,27] A second cytokine, IFN-α, was also used for this disease, with a modest response rate (RR) of approximately 15% as a single agent. Overall, the initial era of immunotherapy for mRCC was characterized by low rates of disease control and high rates of toxicity.

In 2005 and 2006, the Food and Drug Administration (FDA) approved sorafenib and sunitinib and subsequently five other antiangiogenic drugs were approved (pazopanib, axitinib, bevacizumab, cabozantinib, and lenvatinib). Two mTOR inhibitors, temsirolimus and everolimus also showed benefit in randomized, phase 3 trials and were also approved by the FDA.[28] Second-line trials of FDA-approved agents are summarized in Table 1.

Second-line or later treatment for advanced clear cell RCC								
Agents	**Patient *nº***	**Median PFS *month***	**HR for Disease Progression (95%CI)**	**P value**	**Median OS**	**HR for Death (95%CI)**	**P value**	**Objective Response Rate *percent***
Motzer et al.								
Everolimus	272	4.9			14.8			1.8
Placebo	138	1.9	0.33(0.25-0.43)	<0.001	14.4	0.87(0.65-1.15)	0.16	0
Escudier et al.								
Sorafenib	451	5.5			17.8			2
Placebo	452	2.8	0.44(0.35-0.55)	<0.01	15.2	0.88(0.74-1.04)	0.15	0
Rini et al.								
Axitinib	361	6.7			20.1			19
Sorafenib	362	4.7	0.66(0.55-0.81)	<0.001	19.2	0.97(0.80-1.17)	0.37	9
Motzer et al.								
Lenvatinib/everolimus	51	12.8	0.45(0.22-0.79)	0.003	25.5	0.51(0.3-0.88)	0.02	35
Lenvatinib	52	9.0	0.62(0.37-1.04)	0.12	19.1	0.68(0.41-1.14)	0.12	39
Everolimus	50	5.6	-		15.4	-		0
Choueiri et al.								
Cabozantinib	330	7.4			21.4			17
Everolimus	328	3.9	0.51(0.41-0.62)	<0.001	16.5	0.66(0.53-0.83)	0.003	3

Motzer et al.								
Nivolumab	406	4.6			25.0			25
Everolimus	397	4.4	0.88(0.75-1.03)	0.11	19.6	0.73(0.57-0.93)	0.002	5

The response of some patients to immunomodulatory cytokines and the rare spontaneous regressions of metastatic disease provided the rationale for other immunologic approaches for the treatment of mRCC. PD-1 is an immune-inhibitory receptor that belongs to the CD28/CTLA-4 family and is inducibly expressed on CD4+ and CD8+ T cells, natural killer (NK) cells, B cells and monocytes within 24 hours from their immunological activation. [29] Usually, activated T cells, B-cells, NK cells, dendritic cells (DCs) and monocytes express PD-1 to restrict autoimmunity during inflammatory states such as infections. However, tumors have evolved the ability to express the main PD-1 ligand (PD-L1) to exploit this mechanism, thus downregulating the antitumor T cell response.[30] Therefore, inhibiting this pathway with monoclonal antibodies (mAbs) targeting either PD-1 or PD-L1 can reenergize exhausted T cells downregulated by tumor-directed PD-L1 expression, culminating in innate antitumor detection and coordinated tumor cell death.

After promising results in early-phases clinical trials on nivolumab, the Phase III CheckMate-025 trial demonstrated superior median overall survival (OS) with nivolumab (n = 406) in patients with advanced RCC who progressed on antiangiogenic therapy compared with patients receiving everolimus (n = 397; 25 vs 19.6 months, respectively; HR: 0.73; p = 0.002).[31] Nivolumab also showed a trend toward improved progression-free survival (PFS); (HR: 0.88; p = 0.11) and fewer patients experienced grade ≥ 3 adverse events (19 vs 37%, respectively). Based on this trial, nivolumab earned approval by the FDA as a second-line therapy following antiangiogenic treatment failure in patients with advanced RCC [20].

Another immunomodulatory approach studied for the treatment of mRCC is allogeneic hematopoietic stem cell transplantation (allo-SCT) with the hope to induce immune-mediated graft versus-tumor (GVT) effects. In 1999, Childs *et al.* reported the first successful use of non-myeloablative allogeneic transplantation for the treatment of RCC.[32] In 2000, we reported findings from a series of 19 patients with advanced RCC treated with this approach.[33] All 19 patients had had progressive disease before the transplantation, and prior cytokine therapy had failed to produce a response in 17 patients. Stem cells from human leukocyte antigen (HLA)-matched sibling donors were used for 17 patients, and the other two were given cells from a sibling donor mismatched at a single HLA locus. The overall response rate (ORR) was 53%, with three complete and seven partial responses. Responses were seen only among patients with clear-cell carcinomas. The onset of tumor response was delayed by a median of 4 months after transplantation; responses typically appeared after complete donor chimerism had been achieved, after withdrawal of immunosuppressive therapy, or after the development of GVHD. Based on our experience and other investigators experiences,[33,34] allogeneic hematopoietic stem cell transplantation for metastatic RCC was established to be capable of inducing disease regression and sometimes disease remission in patients with advanced RCC, suggesting the presence of GVT effects. Hematopoietic stem cell transplantation can be toxic and associated with a 10-20% risk of procedure-related mortality which greatly limits its application. However, transplant trials showing GVT effects against this malignancy again highlight that ccRCC is an immune responsive tumor where long-term responses can be observed in a small fraction of patients.

Tumor-associated antigen in humans

Tumor cells can express certain molecules, termed tumor-associated antigens (TAAs), which can be used to discriminate tumor cells from most normal tissues. These antigens can be intact on the cell surface or, in the case of proteins, peptide fragments bound to HLA. Among the first HLA-restricted TAAs identified were the melanoma-associated antigen recognized by T cells 1 (MART1), tyrosinase complex and GP100 (also known as PMEL), and T cells reactive with these antigens could be found in tumor infiltrating

lymphocytes (TILs) that were used successfully in adoptive cell transfer (ACT) for patients with melanoma.[35,36] In these studies, effective TILs could not be isolated from all patients, but tumor-reactive T cells could be generated from peripheral blood lymphocytes (PBLs) through genetic modification of TCR genes that were isolated from effective TILs.

Relevant to our study is that some cancers associated with transforming viruses can express viral products, which are attractive targets because they are not expressed by normal tissues. Prior studies indicated that oncogenic proteins encoded by cancer-associated viruses, such as those derived from Epstein–Barr virus[37] and human papillomavirus (HPV)[38] can be recognized by TCR Gene Engineered T Cells. Pre-clinical data also support that the successful targeting of mutant tumor-associated epitopes, especially those derived from driver mutations or transforming oncogenes, can induce regression of cancers.[39]

The goal of adoptive T-cell therapy is to generate a robust immune-mediated antitumor response through the *ex vivo* manipulation of T cells. This aim can be accomplished through the selection and expansion of tumor-infiltrating lymphocytes (TIL), or through gene transfer of a synthetic TCR (TCR) or a chimeric antigen receptor (CAR) into T cells.

Clinical experience with TCR-modified T cells

T cells genetically engineered to express novel receptors can have enhanced tumor specificity and advances in *ex vivo* expansion enable the production of clinically relevant doses of these therapeutic cells. Several TCR clinical trials have been initiated in the US and Europe for the treatment of hematologic and solid cancers. For example, a TCR trial completed in 2015 targeted a human leukocyte antigen HLA-A2–restricted epitope derived from NY-ESO-1, a CGA located on the X chromosome.[40] In this study, more than half of the patients with metastatic melanoma had evidence of cancer regression. Four patients achieved complete remissions, three of which were ongoing after >36 months. An additional 18 patients with synovial cell sarcoma were treated in this same study with also favorable clinical results.

Other trials in this fast-growing field have subsequently demonstrated significant and prolonged tumor regression in patients with melanoma or sarcoma using genetically modified TCRs directed against MART1, melanoma-associated antigen 3 (MAGE-A3), gp100 and carcinoembryonic antigen (CEA). [41-44] Similarly, a TCR trial targeting an A2-restricted epitope derived from the E6 oncoprotein of the high-risk HPV-16 serotype has also been completed in patients with HPV-16+ malignancies (NCT02280811).

Chimeric antigen receptor (CAR) T cells for solid tumors

The demonstration of clinical efficacy in trials using CAR-T-cell therapy is, at present, limited to hematological malignancies, but this modality is being explored clinically in the treatment of solid tumors. Solid tumors present three unique challenges not seen in, for example, B-cell acute lymphoblastic leukemia (B-ALL). Firstly, when compared with B-ALL, their microenvironment can be considerably more immunosuppressive. Secondly, antigen selection is, in general, more difficult because the antigen heterogeneity across the same malignancy is generally higher in solid tumors [45,46]. Thirdly, 'on-target, off-tumor' toxicity is more problematic because potential target antigens in solid tumors are more likely to be expressed in other essential organs. New targets for solid tumors that are beginning to enter clinical studies include mesothelin for the treatment of mesothelioma [47,48], pancreatic[49,50] and ovarian cancer[50]; disialoganglioside GD2[51,52] and EGFRvIII[53] for CNS malignancies; and mucin-16[54] for the treatment of ovarian cancer.

Risk of T-cell therapy

The infusion of T cells is generally well tolerated. Infusional adverse events are infrequent and mild, and are mostly due to the cryoprotectant, dimethyl sulphoxide, or concomitant medication.[55] The main concern of T-cell therapy is the potential for delayed side effects. This became evident from the early days of allogeneic bone marrow transplantation when T cells were recognized as the central mediators of graft-versus-host disease (GVHD).[56,57] Donor T-cell infusion in patients with post-transplant relapse can bring about disease remission through a graft-versus-leukemia effect but this is generally associated with the development of GVHD as a result of alloimmunity against non-hematopoietic tissues.[58,59] Although the antigenic targets in adoptive T cell therapy are much better defined, the potential for adverse effects, both on-target and off-target, remains.

- **On-target but off-tumor adverse effects**

T cells targeting differentiation antigens can be expected to also recognize nonmalignant cells that express the same antigens, resulting in adverse events. For example, melanoma patients treated with T cells targeting melanocyte differentiation antigens, such as MART-1 and gp100, often developed vitiligo and uveitis. These on-target toxicities have been observed across all forms of T-cell therapeutic approaches, including tumor-infiltrating cells,[60] *in vitro*-expanded T-cell clones,[61] and TCR-transgenic cells.[42] In general, on-target autoimmunity is associated with tumor regression,[60,61] and is more prominent in treatment approaches that are more efficacious. Melanoma patients treated with T cells transduced with a high-avidity MART-1(27-35) TCR and murine-derived gp100 TCR had a significantly higher rate of tumor response than an earlier cohort of patients treated with T cells transduced with a lower-avidity MART-1(27-35) TCR.[42] T cells with gene-engineered TCRs specific for PMEL or MART1 mediated the destruction of normal melanocytes in the skin, eyes and ears of treated patients, and these detrimental effects sometimes required intervention with corticosteroid treatment.[42] Not unexpectedly, the rate of autoimmunity was also correspondingly higher: of the 20 patients treated with the high-avidity TCR transgenic T cells, 11 (55%) developed uveitis and 10 (50%) developed a transient steroid-responsive hearing loss; the latter was not observed in previous studies and was attributed to the presence of melanocytes in the striae vascularis of the inner ear.[42]

A similar pattern was seen in CD19 CAR T-cell therapy, where better disease response was associated with long-term B-cell depletion and hypogammaglobulinemia.[62,63] On-target but off-tumor toxicities can be immediately life-threatening. A patient with colorectal cancer with lung and liver metastases developed respiratory distress within 15 min of HER2-specific CAR T-cell infusion and subsequently died from multiorgan failure 5 days later.[64] It was postulated that the T cells recognized HER2 expressed by normal lung tissues, leading to the release of inflammatory cytokines, pulmonary toxicity and a cascading cytokine storm that cumulated in multiorgan failure. This adverse event was not foreseeable as it has not been observed in HER2 vaccine trials or the many breast cancer patients treated with the HER2 monoclonal antibody, trastuzumab. It is thought that the fatal toxicity was a function of the high potency of the CAR construct that contained CD28 and 4-1BB co-stimulatory molecules, and the use of prior nonmyeloablative chemotherapy that further enhanced treatment effect.

On-target toxicities that are not immediately life-threatening can still be treatment-limiting. Carbonic anhydrase-IX (CAIX)-specific CAR T cells, which were studied in patients with metastatic RCC, were associated with dose-limiting liver toxicity because of low-level CAIX expression in bile duct epithelium.[65,66] Similarly, a study using T cells transduced with a high-avidity murine TCR against human carcinoembryonic antigen (CEA) in patients with metastatic colorectal carcinoma was halted after all three patients developed severe transient colitis caused by the recognition of normal levels of CEA in the colonic mucosa.[41] The hepatitis and colitis in both studies were either self-limiting or responsive to corticosteroids and there were no treatment-related deaths.[41,65,66] More recently, however, fatal on-target toxicities were

reported in patients treated with anti-MAGE-A3 TCR-transduced T cells.[44] In this study, HLA-A*0201 transgenic mice were immunized with a MAGE-A3 peptide epitope to generate a high-avidity TCR that recognized not only MAGE-A3 but also MAGE-A9 and MAGE-A12. Nine patients with various malignancies were treated on this protocol and three developed altered mental status within a few days, two of whom became comatose and died. Autopsy showed necrotizing leukoencephalopathy with extensive white matter defects associated with CD8+ T-cell infiltration, caused by the previously unrecognized low-level expression of MAGE-A12 in human brain.[44] In each of these cases, the adverse effects occurred despite relatively low levels of antigen expression in the off-tumor sites, thus highlighting the potential for harm in using redirected T cells with high avidity and potency.

- **Cytokine release syndrome**

Cytokine release syndrome (CRS), which is characterized by fevers, rigors, hypotension and hypoxia, has been observed in a number of CD19 CAR T-cell studies as a result of large-scale T-cell activation upon the recognition of CD19+ malignant cells.[62,63,67,68,69] The symptoms usually begin a few days following T-cell infusion but can be as early as 24 h, depending on the co-stimulatory domains, and coincide with the *in vivo* expansion of CD19 CAR T cells and the elevation of a number of serum cytokine levels, including interferon-γ, soluble interleukin-2 receptor α, interleukin-2, interleukin-6 and tumor necrosis factor. [67-70] Some patients also develop features of macrophage activation syndrome, including very high ferritin levels, histological features of hemophagocytic lymphohistiocytosis, hepatosplenomegaly and disseminated intravascular coagulation.[71]A significant proportion of patients develop alarming but reversible neurological symptoms, including delirium and seizure-like activity, the reason for which is not fully understood but thought to be related to generalized T cell-mediated inflammation rather than direct toxicity of CAR T cells on the brain.[62,67-69] In general, patients with evidence of persistent disease at the time of T-cell infusion are more likely to develop CRS.[69,72]

- **Off-target adverse effects**

In mid-2011, a patient with metastatic melanoma suffered a sudden cardiac death 4 days after an infusion of autologous T cells transduced with an affinity-enhanced HLA-A1-restricted MAGE-A3 TCR.[73] This high-affinity TCR was generated by introducing mutations into the α chain of a MAGE-A3 TCR that was isolated from another patient from a previous vaccination study. The α chain mutations increased the potency of the T cells against MAGE-A3-expressing targets *in vitro* and *in vivo* while maintaining a high level of specificity *in vitro.*[73] Investigations into the death did not identify any evidence of direct T cell-mediated toxicity and a second patient was enrolled the following year. This patient also suffered a cardiac death 5 days after T-cell infusion and was found to have extensive myocardial necrosis on autopsy.[73] Further investigation using a combination of amino acid substitution and *in silico* screening showed that the MAGE-A3 TCR also recognized a peptide from an unrelated muscle protein, Titin, which is important for the contraction of striated muscles.[74] This off-target toxicity would have been very difficult to predict: Titin expression was undetectable in cardiac-derived primary cell lines and could only be detected in a more elaborate beating myocyte culture system,[74] and the toxicity would not have been detected in HLA-A1 transgenic mouse models either because there was no reactivity against the equivalent mouse Titin peptide.[74] Although this remains the only example of off-target toxicity, the difficulties in predicting such toxicity and the fatal outcome are very concerning. Another potential source of off-target toxicity is the mispairing of transgenic α or β TCR chains with endogenous TCR, which can potentially give rise to TCRs with new specificities and autoreactivity.[75] This has been demonstrated in murine models[76] but has not been observed in human clinical studies encompassing >150 patients to date.[77]

There have been few completed clinical trials published utilizing CARs in solid tumor malignancies. While early trials did not demonstrate long-term cell engraftment nor anti-tumor efficacy, the CAIX trial did lead to reports of unexpected biliary tract toxicity, illustrating the potential risk for acute in vivo on-target, off-tumor effects in patients.[66] This study was stopped due to the advent of competing treatments before reaching therapeutic or maximum tolerated dose.

Two case reports and results of a 19-patient trial have also been published. The first case by Morgan*, et al.* reported on the first in human use of a third generation CAR in solid tumors to treat a patient with refractory metastatic melanoma.[64] Unfortunately, this patient experienced an immediate lethal severe adverse event following infusion of a large number (10^{11}) of HER2 CAR T cells, attributed to off-tumor, on-target toxicity in normal tissues. Another report using the second-generation CAR targeting the same HER2 antigen in patients with refractory metastatic sarcomas was recently published.[78] This trial started at a much lower cell dose, with as few as 10^4 T cells, working up to a maximum dose of 10^8 cells, still 3 logs below the dosage given in the NCI trial. While this trial showed no overt toxicity, it did have some indication of efficacy, with one patient experiencing a partial tumor response revealed by positron emission tomography (PET). Table 2 shows characteristics of selected published trials on TCRs and CARs.

Table 2.

TCRs Studies						
TCR Target	**HLA restriction**	**Tumor histology**	**Patient number**	**RR**	**Toxicity**	**Potential mechanism of toxicity**
MART-1	HLA-A2	Metastatic Melanoma	17	12% (2PR)	None	N/A
MART-1 (+vaccine)	HLA-A2	Metastatic Melanoma	20	30% (6PR)	G3 skin G3 hearing G3 sight	On-target, off-tumor toxicity on melanocytes in skin, eye and inner ear
Gp100	HLA-A2	Metastatic Melanoma	16	19% (1CR)	G3 skin G3 hearing G3 sight	On-target, off-tumor toxicity on melanocytes in skin, eye and inner ear
CEA	HLA-A2	Metastatic colorectal cancer	3	33%	G3 colitis	On-target, off-tumor in normal colon
NY-ESO-1*	HLA-A2	Metastatic melanoma/Synovial cell sarcoma	20/18	55%(3CR)/61% (1CR)	None	N/A
NY-ESO-1	HLA-A2	Multiple Myeloma	20	80%	None	NA
MAGE-A3*	HLA-A2	Metastatic melanoma/Synovial cell sarcoma/Esophageal carcinoma	9	55%	Mental status changes and fatalities (2/3)	Neurotoxicity due to cross-recognition of MAGE-A12 in normal brain
MAGE-A3	HLA-A1	Metastatic melanoma/ Multiple myeloma	2	0%	Fatalities	Cardiac toxicity due to cross-recognition of Titin-1 in heart tissue
MAGE-A4*	HLA-A2	Esophageal cancer	10	0%	None	N/A
HPV E6*	HLA-A2	Cervical cancer	9	33% (2CR)	None	N/A
CARs Studies						
CAR Target	**HLA restriction**	**Tumor histology**	**Patient number**	**RR**	**Toxicity**	**Potential mechanism of toxicity**
Alpha folate receptor	-	Ovarian cancer	14	0%	None	N/A
CD171/L1-CAM	-	Neuroblastoma	6	0%	None	N/A

CAIX	-	**Renal cell carcinoma**	**3**	**0%**	**Biliary toxicity**	**On-target, off-tumor toxicity in bile duct**
GD2	-	**Neuroblastoma**	**11**	**27%**	**None**	**N/A**
IL13Rα2	-	**Glioblastoma multiforme**	**3**	**0%**	**None**	**N/A**
Mesothelin	-	**Mesothelioma/Pancreatic cancer**	**1/1**	**0%**	**None**	**N/A**
HER2/ERBB2	-	**Sarcoma**	**19**	**0%**	**None**	**N/A**
HER2/ERBB2	-	**Metastatic melanoma**	**1**	**NA**	**Fatality**	**On-target, off-tumor toxicity in lung and other normal tissues**

- **Insertional mutagenesis**

The integration of viral vectors proximate to growth-promoting genes can result in the transactivation of proto-oncogenes and malignant transformation. Acute leukemia as a result of insertional mutagenesis (IM) has plagued a number of gene therapy studies for primary immunodeficiency disorders, including X-linked severe combined immunodeficiency,[79,80] chronic granulomatous disease[81] and Wiskott–Aldrich syndrome.[82] IM was also reported in a β-thalassemia patient who was treated with a self-inactivating HIV-1-based vector containing the β-globin gene controlled by its wild-type promoter. The patient was treated with autologous CD34+ cells transduced with a lentiviral vector.[83] Clonal population analysis demonstrated a bias for one hematopoietic clone derived from transduced cells with the proviral cassette integrated into the HMGA2 proto-oncogene sequence causing a benign cell expansion. Interestingly, HMGA2 was overexpressed in myeloid cells, but the deregulation was not found in granulocyte-monocyte cells sharing the same vector integration pattern. A recent report in one patient with chronic lymphocytic leukemia (CLL) treated with CARs showed that there may be IM of T cells via lentiviral integration that impact on behavior CAR-T cells, with a TET2 insertion resulting in clonal expansion, direct tumor cell recognition and killing, and persistence of the T cell clone.[84]

In contrast, there has not been any report of insertional mutagenesis arising from gene-modified T cells that have been administered to hundreds of patients. This vast difference in genotoxicity profile may be related to the nature of the transgenes involved and the pluripotency of hematopoietic stem cells, which may render them much more susceptible to malignant transformation than mature T cells.[85]

Targeting endogenous retroviral elements

Approximately 8% of the human genome consists of human endogenous retroviruses (HERVs) that are remnants of ancient infections and incorporation of viral sequences into the germline.[86] The HERVs are mostly transcriptionally inactive due to epigenetic changes,[87] but may be re-activated in tumors and can elicit anti-tumor immunity.[88] There is pre-clinical evidence that TLR7 or RAG knockouts in mice develop uncontrolled endogenous retrovirus expression, endogenous retrovirus infectivity and endogenous retrovirus insertion-driven tumors.[89,90] HERVs have been evolutionary inactivated by accumulation of indels and promoter methylation, but some HERVs have been shown to be transcriptionally active in human malignancies including melanoma[91,92], breast cancer[93], prostate adenocarcinoma[94], ovarian cancer[95], and most recently by our group in ccRCC.[96,97]

Discovery and characterization of CT-RCC HERV-E expressed in ccRCC

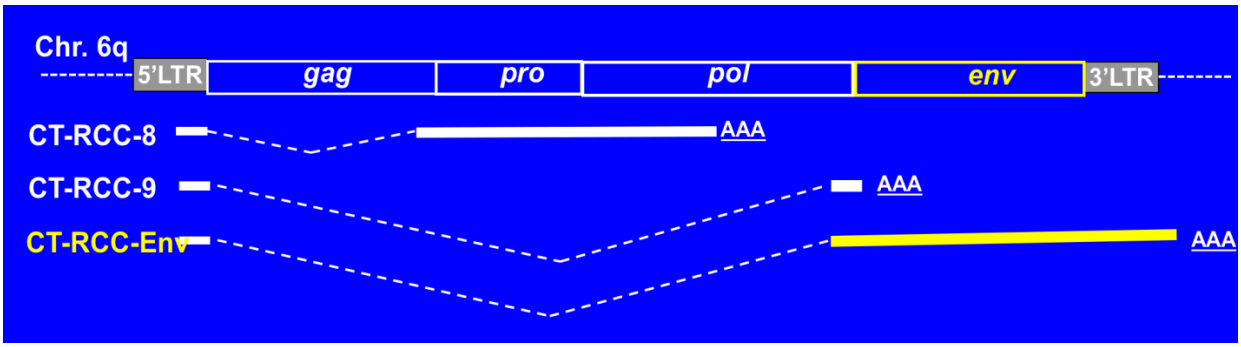
Childs and colleagues examined peripheral blood mononuclear cell (PBMC) from ccRCC patients treated with allo-HSCT, who experienced tumor response following allo-HSCT. The aim was to identify tumor-

restricted antigens that may be involved in T cell-mediated antitumoral activity. As result of this pre-clinical work, cytotoxic T lymphocytes (CTLs) that killed patient tumor cells *in vitro* were isolated from patient's PBMC collected after transplant. These RCC-reactive CD8+ T cells were undetectable in the PBMCs of both the donor and the patient before transplantation.[96] Utilizing cDNA expression cloning, an HLA-A11–restricted 10-mer peptide (named CT-RCC-1) was found as a target antigen for RCC-specific T cells in a number of patients. This peptide (ATFLGSLTWK) was found to be encoded by a novel human endogenous retrovirus (HERV) type E (named CT-RCC HERV-E).[96] At present, three different HERV-E transcripts have been identified, named CT-RCC-8, CT-RCC-9 and CT-RCC-Env (*e.g.*, GenBank Accession Nos. EU137846, EU137847, and JQ733905). Importantly, these transcripts were found to be expressed only in ccRCC cells, but not in other tumors or normal tissues.[7, 9]

A recent report by Rooney *et al*. of the molecular and genetic properties of tumors that had associated local immune cytolytic activity using solid tissue tumor biopsies revealed numerous instances of endogenous retrovirus being reactivated in tumors. In the same study, three tumor–specific endogenous retroviruses, all with minimal to undetectable expression in normal tissues and elevated expression in tumor tissues, were described. One of them was observed in ccRCC tumors (referred as ERVE-4) in strong correlation with local high immune cytolytic activity.[98] The ERVE-4 locus is the same as CT-RCC HERV-E described previously in our lab, but it was named in accordance with other nomenclature in Rooney *et al*. paper. In the following link https://www.ncbi.nlm.nih.gov/gene/?term=ERVE%E2%88%924 you will find evidence that ERVE-4 and CT-RCC HERV-E represent the same HERV-E locus on chromosome 6q. Therefore, the findings by Rooney et al. represent confirmation of our group's findings on the potential of this HERV-E to serve as an excellent target for T cell based immunotherapy trials.

The CT-RCC HERV-E transcripts represent spliced variants sharing common region from 5'LTR and sequences encoding for parts of the protease and polymerase as well as the entire envelope genes, respectively (GenBank accession numbers EU137846.1, EU137847.1, and JQ733905).[99] Sequence analysis of the envelope transcript revealed long open reading frames partially encoding putative surface and transmembrane envelope proteins.

Figure 1.



Inactivation of the VHL tumor suppressor leads to selective expression of a human endogenous retrovirus in ccRCC

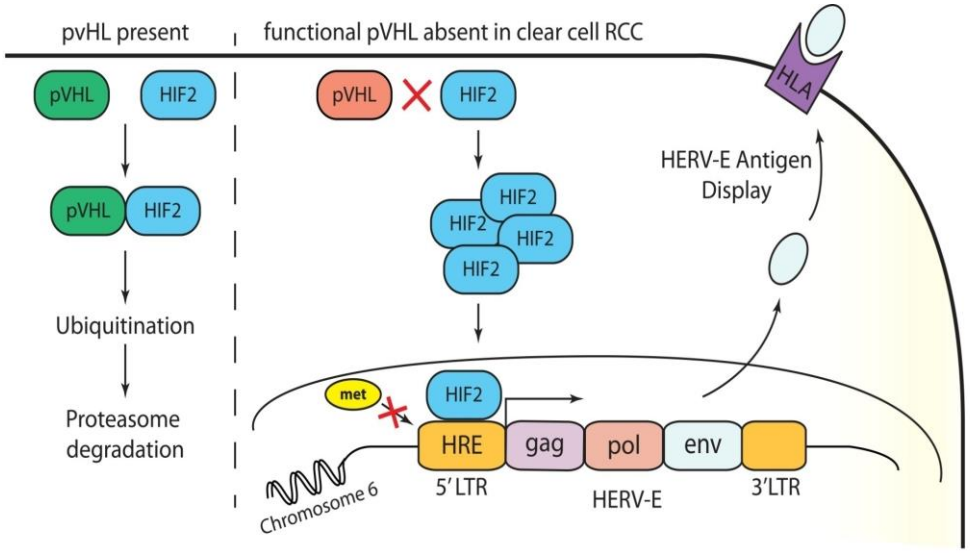
In an attempt to further understand the mechanisms leading to specific expression of the CT-RCC HERV-E in ccRCC, a total of 50 fresh (primary) ccRCC tumors (17 patients with sporadic ccRCC and 23 patients

with *VHL* disease) and 17 non- ccRCC tumor samples and cell lines were analyzed for CT-RCC HERV-E expression and status of *VHL* tumor suppressor gene.

Although expression levels varied among tumors, most ccRCCs (80%) were found to express CT-RCC transcripts. HERV-E expression was found to be restricted to clear cell histological type of RCC and caused by inactivation of the VHL tumor suppressor gene and HIF-2α transcriptional factor stabilization. Cherkasova, *et al*. demonstrated that HIF-2α may serve as transcriptional factor for the CT-RCC HERV-E by binding with HIF response elements (HRE) localized in proviral long terminal repeat (LTR). Remarkably, all ccRCC tumors expressing CT-RCC HERV-E possessed hypomethylated LTR. In contrast, non-ccRCC tumors and normal tissues had a highly methylated proviral LTR preventing CT-RCC HERV-E expression. These findings suggest loss of function of the VHL tumor suppressor and HIF-2α stabilization together with epigenetic modifications lead to the selective expression of this endogenous retrovirus encoding antigens that serve as targets for T cells in ccRCC.[97] (Figure 2)

Our group also demonstrated that exposure of HERV-E-negative ccRCC lines containing a hypermethylated 5'LTR region to the demethylating agent DAC and the histone deacetylase inhibitor DP could enhance the provirus expression *in vitro*. [97] Although the mechanisms involved in deregulation of DNA methylation are not totally understood, our data suggest that treatment of ccRCC patients with demethylating agents would potentially lead to higher levels of CT-RCC HERV-E expression in kidney tumors.

Figure. 2

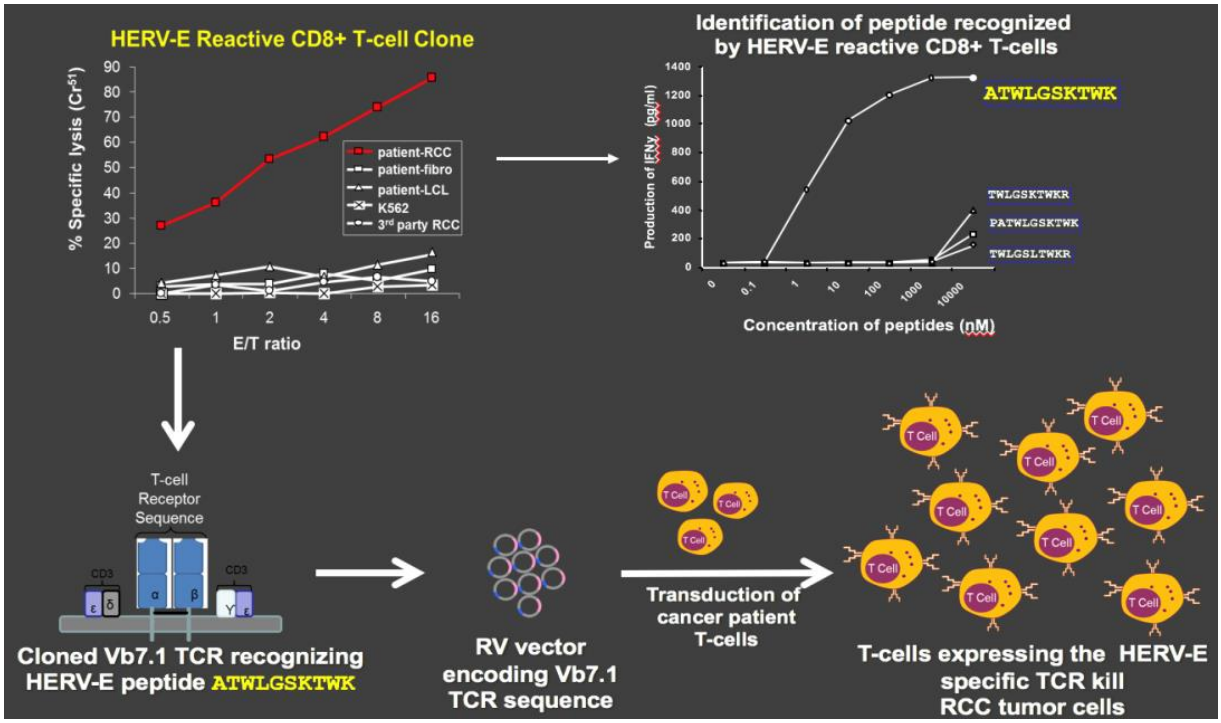


Our findings also concur with current evidence that cellular transformation by endogenous retroviruses-encoded antigens is typically accompanied by major alteration of the epigenetic landscape that is normally found in healthy cells. As result, endogenous retrovirus elements are no longer fully transcriptionally repressed and adaptive immune responses to epitopes derived from endogenous retroviruses are frequently detected in the cancers.[100,101]

In summary, we consider CT-RCC HERV-E to be an exciting new target for ACT for several reasons. First, HERV-E is expressed by ~80% of the ccRCC fresh tumors and established cell lines.[96] Second, CT-RCC HERV-E expression confined to the ccRCC cells with no expression found in other tumors or normal tissues.[96,57,58] Finally, its expression is regulated by inactivation of VHL tumor suppressor gene which is inactivated by mutation or epigenetic changes in the vast majority of ccRCC. [12,102]

Two decades ago, it was demonstrated that it is possible to redirect the specificity of human T cells by engineering them to express TCR genes.[103] However, there were no TCRs cloned that were specific for an antigen expressed by ccRCC and thus, no ACT options existed for such patients. Based on the identification of the antigenicity of the HERV-E transcripts in ccRCC, our team in collaboration with Dr. Nishimura's laboratory at Loyola University Cardinal Bernardin Cancer Center has cloned and characterized a TCR that recognizes HLA-A11-restricted HERV-E-derived antigen CT-RCC-1. To bring this discovery from bench to bedside, our team has already generated two high titer retroviral producer clones which make virus capable of transferring the HERV-E TCR to normal human T cells. We have also created two Master Cell Banks and completed the Good Manufacturing Process (GMP) qualification process. Results of the pre-clinical studies are described in detail in next section. Figure 3 below summarizes the steps taken to develop this immunotherapy.

Figure 3.



HERV-E TCR transduced autologous T cells

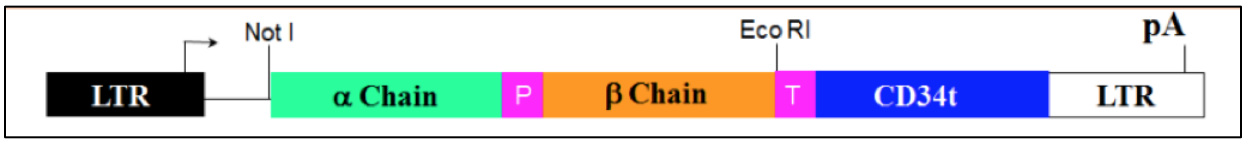
- **Identification of the HLA-A11 restricted TCR sequence specific for CT-RCC-1 antigen and construction of retroviral vectors capable of transferring this TCR to normal PBL-derived CD8+ T cells**

Utilizing a RACE-PCR of total RNA extracted from a CTL clone followed by sequencing, we have identified the sequence of TCR recognizing CT-RCC-1 antigen. Full length cDNAs for α and β chains were cloned into SAMEN retroviral vector (Figure 4). The SAMEN CMV/SRα retroviral vector was designed specifically for introducing TCR genes into alternate T cells. The 5' long terminal repeat (LTR) in the SAMEN SRα backbone was replaced with a hybrid LTR consisting of human CMV enhancer and promoter fused to the MMLV 5' LTR. This modification permits production of transient retroviral supernatants in 293 GP cells. Other key elements of the SAMEN CMV/SRα vector include an internal SRα promoter to permit the expression of multiple genes, unique *Sal*I and *Xho*I restriction sites for ease of inserting TCR chains, and an internal ribosome entry site/neor cassette for G418 resistance.[104] Our collaborators and others in the Surgery Branch, NCI used it for in vitro studies and clinical trials. However, Dr. Nishimura modified it to remove the IRES/neo^r cassette since we no longer use G418 for selection.

The retroviral vector, HERV-E TCR CD34t 7G1, consists of 7,948 bps including the 5' LTR from the murine stem cell virus (promoter), packaging signal including the splicing donor (SD), splicing acceptor sites, alpha chain and beta chain genes of the anti-HERV-E TCR. The HERV-E TCR is also linked to a truncated CD34 chain (CD34t) which was obtained from Dr. Timothy Clay when he was at Duke University. Its origin is human. We used PCR to link the α and β TCR chains together with a T2A self-cleavage sequence to facilitate high and equal expression of both TCR chains. The HERV-E TCR is linked to a CD34t by a different self-cleavage sequence (P2A) resulting in a final transgene cassette consisting of TCR α -TCR β- CD34t. The CD34t protein includes the extracellular and transmembrane regions of CD34, and as a result, it is expressed on the cell surface of transduced cells, but does not affect activity of cells expressing the truncated protein which is normally expressed on hematopoietic progenitor cells. T-cells expressing CD34t can be identified with an anti-CD34 antibody, and can be isolated using flow cytometry or immuno-magnetic methods to purify these transduced T cells and also for subsequent immune monitoring of the genetically modified T cells *in vivo* following adoptive transfer.[105,106] Using this approach, our collaborators routinely isolate >98% pure transduced T cells.

Multiple approaches for inserting TCR genes into T cells by using gammaretroviruses[43,44,60] and lentiviruses [74,107] have been assessed. The backbone of the retrovirus used here is also being used in an ongoing phase Ib gene therapy trial of TIL 1383I TCR transduced T cells in melanoma patients lead by our collaborator Dr. Nishimura at Loyola University (NCT02870244).

Figure 4.



- **Generation of a panel high titer PG13 HERV-E TCR retroviral producer clones and selection of appropriate clones for use in clinical trials**

Once it was established that we successfully cloned a functional HERV-E TCR, the retroviral vector containing this TCR was introduced into the PG13 packaging cell line to isolate high titer retroviral producer clones for clinical use as previously reported.[108] The bulk PG13 producer line was cloned in limiting dilution to generate high titer clones and clones were screened for transduction efficiency based on CD34 expression of the transduced T cells. Then, the clones were screened for their ability to efficiently transduce and transfer anti-HERV-E reactivity to human T cells. Two clones were selected (7G1 and 27A7) for further testing.

- **Evaluation of transduction efficiency, the function of the transduced T cells, and the FDA mandated copy number testing.**

For this purpose, retrovirus was prepared from each clone and two separate full scale validation runs were performed. The Standard Operating Procedures (SOPs) for development of our clinical trial were developed and tested using these two full scale production runs.

An example of one full scale production run is shown in Figure 4. T cells from 2 healthy donors were transduced with retrovirus from clone 7G1 or 27A7. They were 63.2% and 67.5% ($\overline{X}$=65.4%) or 65.2% and 61.6% ($\overline{X}$=63.4%) $CD34^+$ respectively indicating both clones transduced T cells with equal efficiency. Following CD34 purification of the transduced cells using immunomagnetic beads, the cultures were 99.3% and 98.4% ($\overline{X}$=98.9%) or 99.4% and 99.7% ($\overline{X}$=99.6%) $CD34^+$ respectively indicating we had pure TCR transduced T cell cultures. (Figure 5)

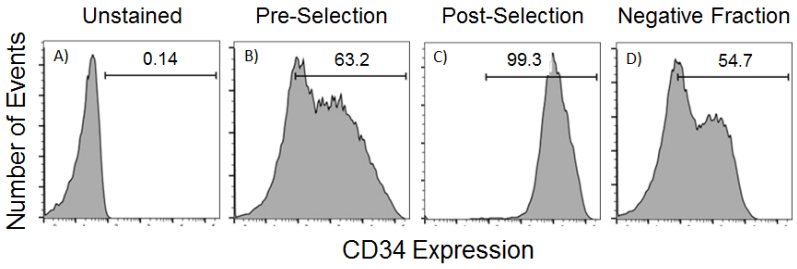


Figure 5. Efficiency of Purifying HERV-E TCR Transduced T Cells. A representative CD34 purification of HERV-E TCR transduced T cells using the CliniMACS is shown. Supernatants from the PG13 retroviral producer clone 7G1 were used to transduce normal PBL-derived T cells using our patient treatment SOP's that will be used on treated patients. Three days post-transduction, the cells were incubated with anti-CD34 coated immunomagnetic particles and purified using the CliniMACS and stained for CD34 expression. The log fluorescence of $>10^4$ live cells were measured by flow cytometry. Unstained (panel A), pre-selection (panel B), the positive fraction (panel C), and the negative fraction are shown.

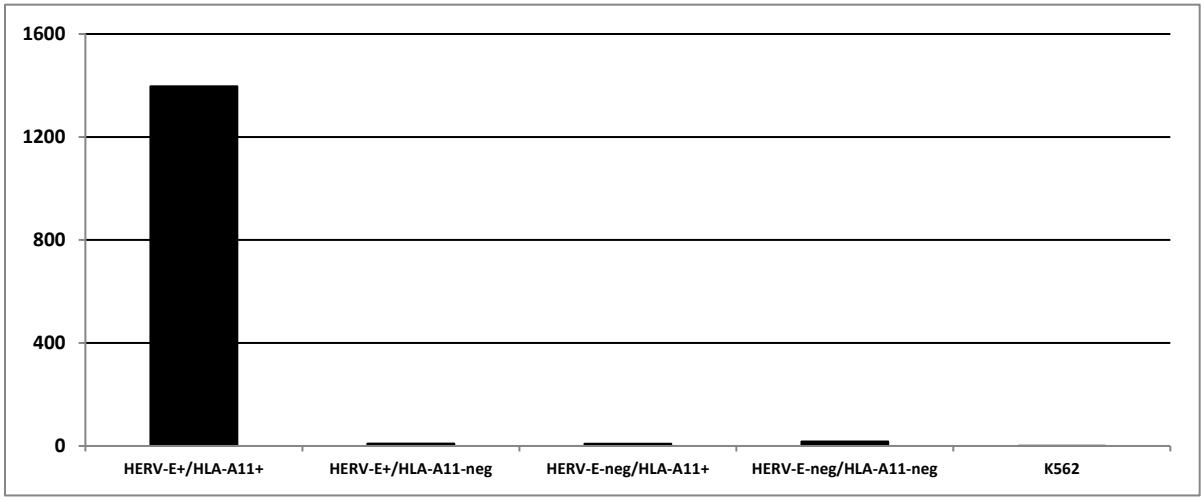
- **Production and GMP qualification of the Master Cell Banks**

We generated a 212 vial PG13HERV-E Master Cell bank from clone 7G1 of $5x10^6$ cells per vial for both clones (212 vials for 7G1 and 220 vials 27A7) in the event one fails GMP qualification. A large 6-liter batch of virus was prepared from the 7G1 clone and both the 7G1 clone and 7G1 virus were sent to the Vector Production Facility/National Gene Vector Biorepository at Indiana University for GMP qualification. Six liters of virus is adequate to complete all GMP qualification studies, the shipping validation runs to the National Institute of Health (NIH) for the clinical trial, and treatment runs for at least 20 patients.

- **Characterization of the reactivity and specificity of HERV-E TCR transduced T cells and determination of the relative affinity of the HERV-E TCR**

We also confirmed that the HERV-E TCR transduced T cells function as predicted. We assessed antigen specificity of HERV-E TCR transduced T cells measuring interferon-γ (INF-γ) secretion by enzyme-linked immunosorbent assay (ELISA) using a panel of ccRCC cell lines as targets. Figure below shows that transduced T cells produce INF-γ only when coculturing with both HLA-A11-positive and CT-RCC HERV-E-expressing ccRCC cells (>twice background and >1 ng/ml).

Figure 6. INF-γ secretion levels after coculture of HERV-E TCR transduced T cells with cancer cells. A representative ELISA assay shows the ability of HERV-E TCR transduced T cells to selectively target ccRCC cells expressing both HLA-A11 allele and the CT-RCC HERV-E provirus. ccRCC cells were incubated at 1:10 effector to target ratio with HERV-E TCR transduced T cells for 18 hours. K562 cells were included as targets to control for NK reactivity. Axis Y shows IFN-γ secretion levels in pg/ml.



HERV-E TCR transduced T cells also lyse an HLA-A11:01^{+}/HERV-E^{+} tumor and an HLA-A11:01^{+}/HERV-E^{-} tumor cells loaded with the CT-RCC-1 peptide but not an HLA-A11:01^{+}/HERV-E^{-} or an HLA-A11:01^{-}/HERV-E^{-} tumor cells. The killing was not NK cell-mediated because K562 cells were not lysed. These results support the ability of our HLA-A11-restricted HERV-E-specific TCR to transfer anti-tumor reactivity against ccRCC via targeting of a HERV-E antigen to normal PBL-derived T cells. (Figure 7).

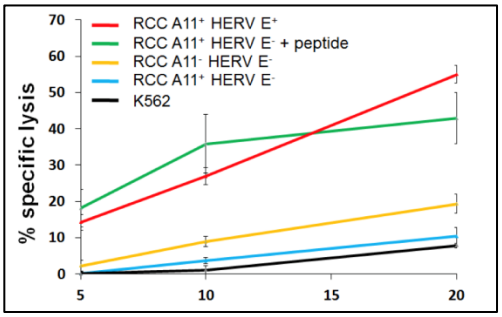


Figure 7. Killing of RCC Cells by HERV-E TCR Transduced T Cells. A representative CTL assay shows the ability of HERV-E TCR transduced T cells to selectively lyse targets expressing the HERV-E antigen . ^{51}Cr labeled RCC lines were incubated at various effector to target ratios with HERV-E TCR transduced T cells. The amount of ^{51}Cr released was measured in a γ-counter and the percent specific lysis was determined. As a positive control, an HLA-A11:01+ HERV-E target loaded with the HERV-E peptide was included (green). K562 cells were included as targets to control for NK lytic activity (black).

An FDA mandated assay required to be performed for our clinical trial is to determine the average number of copies of the integrated virus per cell. The FDA mandates the copy number to be less than 5 for any gene

therapy product being used to treat a patient. We used a PCR-based assay to measure the copy number on days 6 and 10 of culture of ~99% pure HERV-E TCR transduced T cells. On day 6 and 10, the two full scale production runs of T cells transduced with retrovirus from clone 7G1 or 27A7 had less than 5 copies of the integrated virus per cell. These results indicate our cell production SOPs will generate cell products that conform to the FDA copy number requirements.

The frequency of the HLA-A11:01 gene in various U.S. populations is as follows [109] (number of patients with the allele is approx. 2x the frequency minus frequency of homozygosity at the A locus):

Gene frequencies of HLA-A alleles in US populations					
HLA-A	**African Americans** (n = 252)	**Caucasians** (n = 265)	**Hispanics** (n = 234)	**North American Natives** (n = 187)	**Asians** (n = 358)
11:01	0.0238	0.0698	0.0577	0.0267	0.2312

Although HLA-A*10:01 is present in around 15% of the population, there are over 15,000 patients in the U.S. who will die of kidney cancer, so we expect grossly 2,000 patients per year in the U.S. to have HLA-A11:01 and ccRCC for which we have identified a specific TCR. Numbers would be much larger should efforts be made to recruit patients with ccRCC from Asia.

Rationale for immunosuppressive chemotherapy and selection of lymphocyte-depleting chemotherapy regimen

Conditioning chemotherapy regimen of cyclophosphamide and fludarabine is planned to be administered before infusions of HERV-E TCR transduced T-cells because substantial evidence demonstrates an improvement of the anti-malignancy activity of ATC when chemotherapy or radiotherapy are administered before the T cell infusions.[110-112] In mice, administering chemotherapy or radiotherapy prior to infusions of tumor-antigen-specific T cells dramatically enhanced the anti-tumor efficacy of a variety of different tumor-reactive T cells.[37,110,111,113,114]

Studies in murine models and humans have defined the mechanisms of action of the lymphodepleting chemotherapy before cell transfer.[115,116] Lymphodepletion has a variety of positive effects including the elimination of T-regulatory cells and/or myeloid-derived suppressor cells and the elimination of endogenous lymphocytes that provide a sink for growth promoting cytokines such as IL-7 and IL-15.[110,111,113,117] In summary, elimination of endogenous “cytokine sinks” by depleting endogenous T cells and natural killer cells induced an increase of serum levels of important T-cell stimulating cytokines such as IL-15 and IL-7, and the enhancement in T-cell function and anti-tumor activity were dependent on IL-15 and IL-7.[110] The lymphodepleting regimen can also lead to activation of antigen-presenting cells, in part by increased susceptibility to toll-like receptor stimulation.[118]

Although pre-clinical and clinical data initially suggested that adding total body irradiation (TBI) to the non-myeloablating chemotherapy preparative regimen for adoptive transfer of tumor infiltrating lymphocytes (TIL) may lead to more favorable clinical outcomes, a randomized study in metastatic melanoma patients receiving adoptive transfer of TILs could not confirm this initial hypothesis.[119] This randomized study tested the impact of the addition of TBI to the preparative lymphodepleting chemotherapy regimen with similar results in clinical outcomes in both arms. Complete responses rates were 24% in both groups (12 of 50 *versus* 12 of 51), and OS was also similar (median OS of 38.2 months *versus* 36.6 months

with a hazard ratio, 1.11; 95% CI, 0.65 to 1.91; P = .71). Based on these results, we opted to use only lymphodepleting chemotherapy without total body irradiation (TBI) as a conditioning regimen in our current protocol.

The chemotherapy regimen that best increases the anti-malignancy efficacy of TCR gene engineered T cells is not known, but the most commonly used chemotherapy regimens that have been used in clinical trials and that convincingly demonstrate persistence and in vivo activity of adoptively transferred T cells have included cyclophosphamide and/or fludarabine.[38,40,120] We will use cyclophosphamide 1,000 mg/m^2 IV x 1 day (day-4) and fludarabine 30mg/m^2 IV daily x 3 days (days -4 through-2) with the aim of achieving maximum lymphodepletion at the day of the HERV-E TCR transduced T-cells infusion.

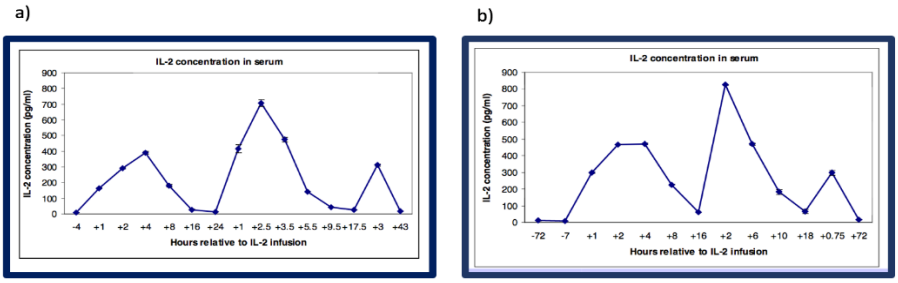
Rationale for dose-escalation of HERV-E TCR transduced T-cells

The clinical trial described in this protocol is designed as a dose escalation study in which the number of HERV-E TCR transduced T-cells administered to patients will be increased with sequential dose levels. The rationale for conducting a dose-escalation trial of a cellular therapy is based on two main sources of evidence. First, the anti-tumor efficacy of adoptively-transferred T cell treatments increases as the dose of T cells administered to mice increases.[121-123] Second, in the setting of allogeneic transplantation, relapsed malignancy is often treated with infusions of allogeneic donor lymphocytes (DLIs).[124,125] The incidence of graft-versus-host disease, which is caused by T cells attacking allogeneic antigens on host tissues, increases as the dose of T cells administered in DLIs increases.[124,125] In this trial, the first dose level has been set at 1 x10^6 HERV-E TCR transduced CD8+/CD34+ enriched T-cells/kilogram, which is less than a third of the dose that was established to be tolerable on protocol NCT02870244.

Rationale for IL-2 regimen after HERV-E TCR transduced T-cells infusion

Additional improvements can be achieved by the systemic administration of interleukin (IL)-2 or other cytokines to support the transferred cells.[122,126] The use of IL-2 to grow T cells in vitro that can be used for ACT, as well as the administration of IL-2 to support the growth and survival of antitumor cells infused into patients, has been a major translation from basic studies of IL-2 to the clinic.[127-133] Most TIL-ACT protocols have used HD bolus IL-2 regimen and the efficacy of this three-step treatment (lymphodepleting chemotherapy, T cell infusion and HD-IL2 administration) has been proven in several phase II trials.[41,43,44,134-137] The toxicities associated with high-dose (HD) bolus IL-2 classically administered after T cell infusion are severe and represents a major limitation for a more widespread use of ACT. Because of this, various doses and schedules of IL-2 administration were evaluated in the previous decades in an attempt to reduce toxicity without compromising efficacy. Different doses and schedules of IL-2 administered in this context have been explored resulting also in long-lasting complete responses in patients with treatment-refractory melanoma.[138,139] Although transient, the toxicities associated with HD IL-2 classically administered together with TILs and TCRs therapies are often severe leading to premature discontinuation of the exogenous administration of IL-2 .[38,134,140] For example, of the 15 planned doses of HD IL-2 to be administered to patients being treated with human papillomavirus–targeted tumor-infiltrating T cells, patients were only able to receive a median 5 (range 0-9) doses due to IL-2 toxicities .[38] Furthermore, no convincing evidence exists to date between the number of doses of IL-2 administered and the efficacy of most adoptive T-cell based immunotherapies.[134,140] Therefore, in the current study, we have opted to utilize an intermediate dose of IL-2 of 2 million units/M2 IV q 12 hours x 14 doses following the T-cell infusion. Prior research conducted by our group in patients receiving the same dose of IL-2 given subcutaneously followed adoptive infusion of ex vivo expanded NK cells (NIH Protocol 08-H-186) have established this dose to be well tolerated, to not be associated with any grade 4 toxicities, and capable of maintaining IL-2 serum level troughs of at least 100 pg/ml (Figure 8 below- data from Protocol 08-H-0186)

Figure 8. Serum levels of IL-2 based on daily (a) versus every 12-hour (b) dosing



3. SCIENTIFIC AND CLINICAL JUSTIFICATION

3.1 Study Design

This is a single-arm, phase 1 trial of HERV-E TCR transduced CD8+/CD34+ enriched T cells in HLA-A*11:01 positive patients with metastatic ccRCC. The study is planned based on a Phase 1 "3+3" dose escalation design. The maximum tolerated dose (MTD) is defined as the highest dose at which 0 or 1 patient in six has experienced a dose limiting toxicity (DLT). Patients with evaluable advanced/metastatic ccRCC will be recruited in up to 4 dose levels. The decision to escalate, de-escalate or suspend the dose escalations in the study will follow the rules outlined in the Table 3.

Table 3.

Outcome: No of DLTs out of No. of Patients at a Given Dose Level	Decision Rule
0 DLT out of 3 patients	Enter up to 3 patients at the next dose level
2 DLTs out of 2-3 patients	Stop dose escalation: Enter up to 3 additional patients at the previous dose level if only 3 patients have been treated at that dose.
1 DLT out of 3 patients	Enter up to 3 more patients at the same dose level .
1 DLT out of 6 patients	Enter up to 3 patients at the next dose level
2 DLTs out of 4-6 patients	Stop dose escalation: Enter up to 3 additional patients at the previous dose level if only 3 patients have been treated at that dose.

Since adverse events associated with adoptive T cell transfer generally occur within 21 days after the T cell infusion, each subject will be observed for 21 days post T cell infusion before the next subject in the cohort or the first subject in the next cohort is treated. Therefore, there will be a minimum of 21 days between the HERV-E TCR transduced T-cell infusion for each patient before the next patient enrolled starts conditioning chemotherapy.

This study will enroll a minimum of 15 patients if no DLTs are observed. If sufficient cells cannot be grown for a patient to meet the criteria for the first dose of cells for the assigned dose level, treatment for that patient on the clinical trial will be aborted. A second attempt will be made to prepare cells for the patient if the patient agrees and if the patient still meets all eligibility criteria. If cell growth limitations still preclude administration of the cohort specified dose, the patient will be taken off study and not receive treatment due to technical failure. If it proves to be technically impossible or impractical to achieve the higher dose levels due to cell production constraints and a maximum tolerated dose has not been defined, the highest achievable dose level will be declared the maximum feasible dose.

3.2 Dose-limiting toxicities

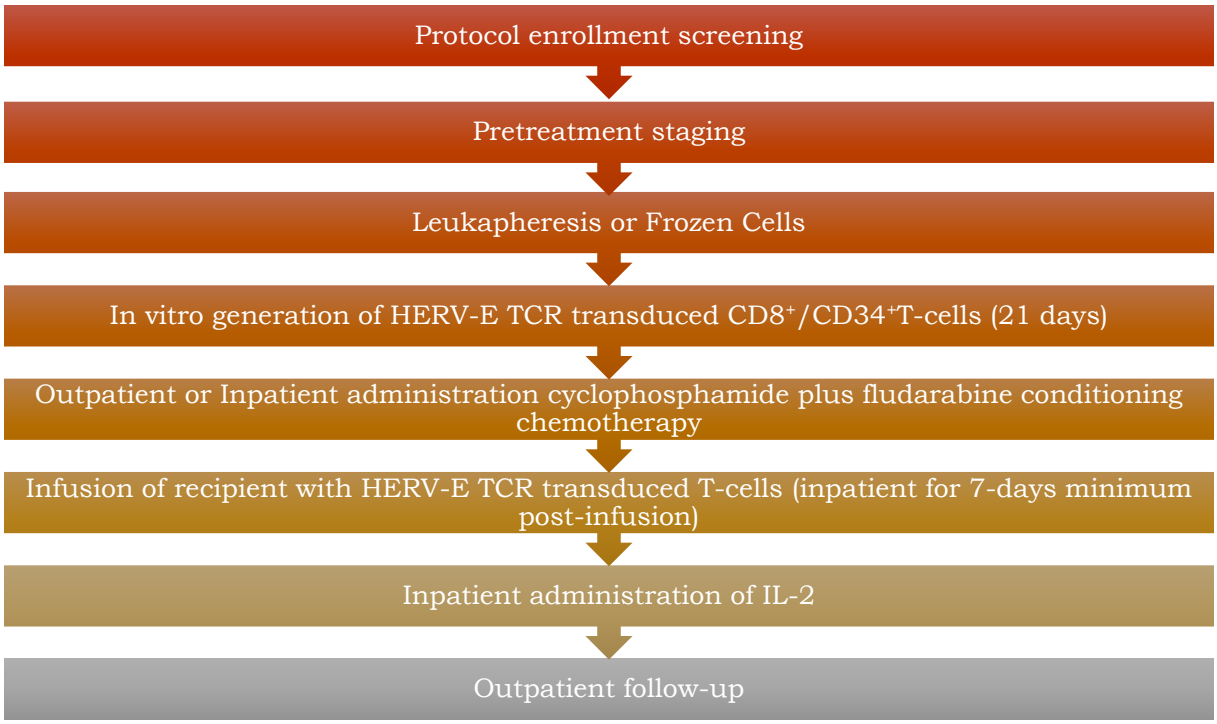
- Because cyclophosphamide, fludarabine and IL-2 are FDA approved drugs with known toxicity profiles at the doses utilized in this protocol, any observed or volunteered grade 3 or 4 adverse events that are as listed on the drug labels will not be counted toward DLT unless the grade 3 or 4 toxicity is previously unknown. Because subjects enrolled on this study may have disease-related or prior treatment-related hematologic toxicity, hematologic DLTs will not be based on CTCAE version 5.0 grading, rather they will be based on recovery of baseline hematologic indices.

- Dose-limiting toxicity is defined as all grade 3 and 4 toxicities judged probably or definitely associated to the HERV-E TCR transduced CD8+/CD34+ enriched T-cells infusion except for:
  - Myelosuppression, defined as lymphopenia, neutropenia and thrombocytopenia.
  - IL-2 expected toxicities. (described section 14.3)
  - Immediate hypersensitivity reactions (excluding symptomatic bronchospasm and grade 4 hypotension) occurring within 2 hours of cell infusion (related to cell infusion) that are resolved within 24 hours of cell administration with standard supportive treatments.
  - Grade 3 Fever.
  - Grade 3 autoimmunity, that resolves to less than or equal to a grade 2 autoimmune toxicity after medical management (e.g., immunosuppressant treatment) within 10 days.
  - Grade 3 metabolic laboratory abnormalities without significant clinical sequela that resolve to grade 2 within 7 days.
  - Hypotension requiring treatment with vasopressors (norepinephrine dose of >2 mcg/minute or equivalent) for 72 hours or less. The 72 hours is measured from the first institution of vasopressors even if vasopressors are temporarily discontinued and then re-started. Norepinephrine doses of <2 mcg/minute or equivalent doses are not a DLT.

- Any adverse event that leads to a discontinuation of the T-cell infusion will be considered a DLT.

If a patient experiences a grade 3-4 toxicity during the cell infusion (reaction to cellular product or infusion reaction) the patient will receive no further cells but may continue to receive IL-2 if the toxicity returns to grade 2 or less within 48 hours. If a patient develops an acute infusion reaction, we will follow a supportive care plan as outlined in Appendix D.

Protocol Schema



All subjects who meet the enrollment criteria and sign the informed consent will undergo a 15-liter (3-4 hour) apheresis at the National Institutes of Health (NIH) Blood Bank to obtain the large number of T cells needed for transduction. The apheresis product will be shipped to the Cellular Therapy Center for processing and TCR transduction to the Cellular Therapy Center at Loyola University Medical Center (LUMC) or another ORSC-approved contracted manufacturing facility for processing and TCR transduction (by World Courier).

3.3 Product manufacturing and testing

After cells are obtained by apheresis at the NIH, the product will be packaged and shipped to the Cellular Therapy Center at Loyola University Chicago, HSD in Maywood IL or another ORSC-approved contracted manufacturing facility for further cell processing to generate HERV-E TCR transduced T cells.

The process to generate the HERV-E TCR transduced T cells is briefly described herein. The PBMCs are isolated from the apheresis product using the cGMP density gradient separation solution Ficoll Paque Premium. Once the PBMCs are isolated, they are washed a minimum of three times with a 0.9% Sodium Chloride solution or DPBS. A sample of the washed PBMCs is inoculated into BD aerobic and anaerobic culture bottles and submitted to the clinical microbiology laboratory for sterility testing.

$2x10^9$ PBMCs are diluted to a concentration of $1x10^6$/mL and activated with 50 ng/mL of soluble anti-hCD3 monoclonal antibody (OKT3) in AIM-V culture media containing 5% human AB Serum, 300 IU/mL rhIL2 and 100 ng/mL rhIL15. A maximum of 200 mLs of cell suspension per one T175cm^2 flask is added to ten (10) T175cm^2 flasks then placed in a humidified 5% CO_2 incubator for 2-3 days.

A large lot of HERV-E retrovirus was previously prepared and cGMP qualified. Briefly, the retrovirus was prepared by seeding T175cm^2 flasks each with $8x10^6$ PG13 HERV-E TCR CD34 T cells from the cGMP qualified PG13 HERV-E TCR CD34t 7G1 Master Cell Bank. The following day, each of the seeded flasks were treated with media containing Sodium Butyrate for 8-10 hours, rinsed with DPBS then 20 mL of

IDMEM+10%FBS media was added for overnight retrovirus production. The retrovirus was collected and filtered with a 0.45µm cellulose acetate filter to remove debris, aliquoted into labeled conical tubes, samples removed for testing and the aliquots snap frozen and stored in the -80ºC freezer located within the Cellular Therapy Center.

On the day of transduction, the activated T cells will be collected, counted and CD4 depleted using the CliniMACS Cell Selector instrument. Post CD4 depletion, $200x10^6$ cells will be transferred to a separate conical tube, pelleted and re-suspended in AIM-V media with 5% human AB serum to a concentration of $4x10^6$ cells/ml. Cytokines (300IU/mL rhIL2, 100ng/mL rhIL15) are added to the cell suspension based on the final total volume of 240mL. 0.5mL of the cell suspension will be added to each well of 24-well non-tissue culture treated plates which have been pre-coated with 30ug/mL clinical grade Retronectin. Aliquots of the cGMP qualified retrovirus will be thawed in a 37ºC water bath and 2mL will be added to each well containing the cell suspension. Each plate is sealed with parafilm, centrifuged at 2000xg, at 32ºC for 2 hours with no brake to bind the retroviral particles and the cells to the retronectin facilitating interaction of the viral particle with the activated T cells. When the spin is complete, the parafilm is removed and the plates are placed in a humidified 5% CO_2 incubator overnight.

The day after transduction, the cells are collected from each well of the plates, transferred to a sterile conical tube, counted, pelleted by centrifugation and re-suspended in fresh complete media containing 300IU/mL-rhIL2 and 100ng/mL-rhIL15 to a concentration of $0.5x10^6$/mL. A maximum of 200mLs of the cell suspension is transferred to each T175cm^2 flask and placed in a humidified 5% CO_2 incubator for culture.

Approximately 3-5 days after the transduction, the transduced T cells are collected and counted. A sample is removed for analysis by flow cytometry. The cells are pelleted and re-suspended in Miltenyi buffer in preparation for CD34 purification. The transduced T cells, which express $CD34^+$ on the cell surface as a transduction marker, are incubated and tagged with Miltenyi GMP biodegradable magnetic micro-particles coated with $CD34^+$ antibody. The magnetic particles bind to the $CD34^+$ molecule present on the cell surface of transduced T cells. The $CD34^+$ cells are positively selected by magnetic separation using the CliniMACs Cell Selector. The $CD34^+$ enriched positive fraction and the CD34 depleted negative fraction is collected and individually transferred to sterile tubes and counted. Samples from both groups are removed for analysis by flow cytometry. The negative fraction is cryopreserved in a 10% DMSO solution. The positive fraction is re-suspended to a concentration of $0.5x10^6$/mL in complete media containing 300IU/mL-rhIL2 and 100ng/mL-rhIL15 and plated in T175cm$_2$ flask (maximum of 200mLs per flask). The flask(s) are placed in a humidified 5% CO_2 incubator for culture. The positive fraction cells are cultured for several days until the start of Rapid Expansion Protocol (REP).

The positive selected $CD34^+$ T cells are placed into a REP to rapidly generate the number of cells needed for infusion. The number of transduced cells that are placed in the REP is determined by the patient's weight and the infusion cohort. To initiate the REP, $1x10^6$ HERV-E TCR transduced cells will be combined with $200x10^6$ feeder cells in an upright T175cm2 flask containing 150mLs complete cytokine media (rhIL2/rhIL15) with 30ng/mL of soluble anti hCD3 antibody (OKT3). The feeder cells consist of PBMC from a minimum of 3 separate normal donors which are mixed together and irradiated with 5000 RAD of irradiation. The flasks are placed upright in a humidified 5% CO_2 incubator for 5-6 days. The flasks are removed from the incubator, the cells collected, counted, and re-suspended in fresh media containing 300IU/mL-rhIL2 and 100ng/mL-rhIL15. The cell suspension is transferred into Wave bioreactor bag(s) for further expansion. The culture media is replenished daily with fresh medium containing cytokines by utilizing the Wave bioreactor perfusion system.

Periodically during the expansion process, samples are removed for microbiology sterility testing and analysis by flow cytometry. The microbiology sterility samples are submitted to the clinical microbiology laboratory in the Loyola Medical University Hospital or another ORSC-approved contracted manufacturing facility in aerobic and anaerobic sterility bottles for 7-14 day sterility testing. The sample removed for flow cytometry is stained for the appropriate markers with fluorochrome conjugated monoclonal antibodies to determine the percent transduction and transgene expressions levels on the transduced T cells.

Approximately 10 days (+/- 2 days) after initiation of the REP, the final infusion product is prepared for packaging and cryopreservation. The HERV-E TCR transduced T cells are collected from the Wave bag, washed, resuspended, counted and a sample removed for flow cytometry to determine the percent $CD8^+CD34^+$ enriched T cells. The number of $CD8^+CD34^+$ enriched T cells needed for infusion, a test sample vial and product testing are transferred to a separate conical tube. The cells are pelleted, the supernatant aspirated and the cells are resuspended in cold cell suspension media to ½ the total packaging volume. ½ the total packaging volume of cold 2x DMSO freeze media is slowly added dropwise to the cell suspension. The cells are well mixed and transferred to the labeled cryopreservation bag. Samples are removed from the final product bag for gram stain testing, endotoxin testing and full sterility testing. The product is cryopreserved to -80°C then transferred to a LN_2 freezer for storage in the vapor phase of LN_2.

Ms. Gina Scurti from Loyola or designee at another ORSC-approved contracted manufacturing facility will be shipping the cryopreserved final product after gram stain, endotoxin and preliminary sterility results are obtained and the QA representative releases the product will be placed in a Princeton Cryo dry shipper (MVE CryoShipper with 4R9953 Blood Bag Rack) and shipped overnight to the NIH in Bethesda, MD using World Courier. These shippers are designed to safely ship cells while maintaining temperatures of -150°C for up to 10 days. The final products will be stored at the NIH Department of Transfusion Medicine (DTM). Infusion of the HERV-E TCR transduced T cells will occur after the 7-day sterility results are obtained and provided to the NIH DTM. The patient materials being sent to Loyola another ORSC-approved contracted manufacturing facility will be identified with patient name and other identifying information to maintain proper chain of custody.

Time	Procedures and Testing
Day 0	**Receive apheresis from NIH** **Sterility Testing** **Perform Ficoll peripheral blood mononuclear cell (PBMC) Isolation** **Stimulate $1.2x10^9$ PBMCs**
Day 1	**Coat Plates with Retronectin**
Day 2	**CD4 Depletion** **Transduction of CD8 cells**
Day 3	**Transfer cells to flasks**
Day 6	**CD34+ Selection on CliniMACs** **Flow Analysis pre/post selection** **Cryopreserve Negative Fraction** **Isolate germline DNA** **Vector Copy Number Assay** **Replication Competent Retrovirus Detection Assay**

Day 8	**Sterility Testing** **Mycoplasma Testing**
Day 10 **REP Day 0**	**Rapid Expansion Program (REP)** **FACs Analysis** **Isolate germline DNA** **Vector Copy Number Assay** **Replication Competent Retrovirus Detection Assay**
Day 15 **REP Day 5**	**Transfer to WAVE** **Elisa** **Sterility Testing** **Mycoplasma Testing**
Day 18 **REP Day 8**	**FACs analysis**
Day 20 **REP Day 10**	**FACs analysis** **Package Infusion Product** **Cryopreserve Infusion Product** **Sterility, Gram Stain** **Mycoplasma** **Endotoxin** **Isolate germline DNA** **Replication Competent Retrovirus Detection Assay** **Sent sample for Full RCR Testing**
Day 21 **REP Day 11**	**Ship Cryopreserved Infusion product to NIH**

Release criteria for the final HERV-E TCR transduced CD8+/CD34+ enriched T cells product are described in Table 4.

Table 4. Test performed on final product prior to release for use from the NIH DTM (frozen product)

Test	Method	Acceptance Criteria
Viability at time of Packaging	Trypan Blue Exclusion	> 70%
Total Viable Cell Number	Visual microscopic count	Minimum Treatment Dose Cell #
$CD34^+$	FACS analysis	>25%
$CD8^+$	FACS analysis	>80%
$CD8^+/CD34^+$	FACS analysis	> 25%
Sterility	Gram Stain	Negative

	BACTEC Aerobic culture	No growth
	BACTEC Anaerobic culture	No growth
	Mycoplasma test	Negative
Endotoxin	Limulus assay	<5 EU/Kg
Vector Copy #	PCR-based assay	<5 copies/cell
RCR by PCR	PCR-based assay	Negative
Interferon Gamma Release	ELISA	2X over background

Once the T cells have been transduced and shown to have met interim lot release criteria, so they are projected to be ready for infusion, patients will receive non-myeloablative lymphocytic-depleting chemotherapy. Cells must be on site at the NIH before conditioning begins with the combination of cyclophosphamide 1,000 mg/m^2 IV x 1 day (day -4) and fludarabine 30mg/m^2 IV daily x 3 days (days -4 through -2). On day 0, subjects will then receive a single infusion of thawed, autologous HERV-E TCR transduced CD8 enriched T cells supported with moderate-dose IL-2 to begin as soon as is feasible after the cell infusion. Although the autologous HERV-E TCR transduced T cells will be enriched for CD8+ T cells, they will consist of a polyclonal mixture of both CD4$^+$ and CD8$^+$ T cells expressing the transduced HLA-A11-restricted HERV-E-specific TCR.

Four cohorts of 3 patients will be treated with increasing doses of HERV-E TCR transduced T cells as shown in Table 5. Since the lot release criteria will require a minimum of 25% of HERV-E TCR transduced CD8+/CD34+ enriched T cells, the actual number of cells infused will be based on patient weight in kilograms with cohort 1 receiving 1 x 10^6 HERV-E TCR transduced T cells per kg body weight, cohort 2 – 5 x 10^6 HERV-E TCR transduced T cells per kg body weight, cohort 3 – 1 x 10^7 HERV-E TCR transduced T cells per kg body weight, and cohort 4 – 5 x 10^7 HERV-E TCR transduced T cells per kg body weight. If the patient has a body mass index (BMI) >35 (i.e. meets criteria for morbid obesity), the weight used to calculate HERV-E TCR transduced T cells doses will utilize a weight derived from the subject's BMI capped at 35. A deviation of ± 10% in cells from the listed doses are acceptable. The weight used to calculate the number of HERV-E cells infused will be the weight on the day of the consent.

Patients will receive intravenous infusions of moderate dose IL-2 (2,000,000 IU/m^2) IV every 12 hours for 14 doses for 7 days post-infusion. Patients will be monitored clinically and immunologically for a year after infusion.

Table 5.

First-dose Escalation Plan

Dose Level	**Dose of HERV-E TCR transduced T-cells**
Level 1	1 x10^6 HERV-E TCR transduced CD8+/CD34+ enriched T-cells per kg body weight
Level 2	5 x 10^6 HERV-E TCR transduced CD8+/CD34+ enriched T-cells per kg body weight
Level 3	1 x 10^7 HERV-E TCR transduced CD8+/CD34+ enriched T-cells per kg body weight
Level 4	5 x 10^7 HERV-E TCR transduced CD8+/CD34+ enriched T-cells per kg body weight

4. ELIGIBILITY CRITERIA

4.1 Inclusion Criteria

- Patients must have histologically confirmed RCC with clear-cell component by the Laboratory of Pathology of the NIH and/or outside Pathology Department prior to entering this study.
- Patients must be HLA-A 11:01 positive (confirmed by HLA typing at the NIH DTM)
- Patients must have measurable disease per RECIST version 1.1 and have disease progression during or after the last treatment regimen and within 6 months before study enrollment
- Patients must have received at least one antiangiogenic drug and an immune-checkpoint inhibitor (i.e. nivolumab unless the patient has contraindications to receiving these medications, the agents are not available to the patient, or the patient declines to receive these drugs due to personal preference.
- Patients must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, the duration of study participation and 180 days (female patients) or 90 days (male patients) after the end of the treatment if sexually active and able to bear or beget children. In addition, male patients must refrain from sperm donation for 90 days after the final dose of investigational product. Female patients must refrain from egg cell donation for 180 days after the final dose of investigational product.
- Patients must be between the ages of 18 and 75 years.
- Patient must have an anticipated life expectancy of at least 3 months.
- Patients must have a performance status of 0 or 1 ECOG performance status (PS) scale (Appendix B).
- Patients must have a caregiver willing to stay with them during the first month of treatment (30 days +/- 7 days).
- Patients receiving treatment with bisphosphonates or denosumab are eligible for enrollment if on a stable dose for ≥4 weeks
- Serology:
  - Seronegative for HIV antibody. (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who are HIV seropositive can have decreased immune-competence and thus be less responsive to the experimental treatment and more susceptible to its toxicities.)
  - 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.

- Organ Function

Hematology	
Absolute neutrophil count	≥500/ µL
WBC	≥1500/µL
Platelet count	≥75.000/µL (without transfusional support)
Chemistry	
Serum AST/ALT	≤ 2.5 x upper limit of normal (ULN)
Total bilirubin	≤ 1.5 mg/dl except for patients with Gilbert's syndrome who must have a total bilirubin ≤ 3 mg/dl
creatinine clearance	≥ 50 ml/min/1.73m² by the method of CKI-EPI or ≥ 50 ml/min by the method of 24h Clearence of Creatinine Calculation
INR	< 1.5
Cardiology	
Estimated left ventricular ejection fraction by echocardiography	≥ 45%
Respiratory	
Predicted DLCO/Alveolar Volume Adjusted by PFT	≥ 45%

4.2 Exclusion Criteria

- Patients that require immediate therapy due to tumor mass effects or spinal cord compression.
- Patients must not have had standard of care anti-VEGFR therapy (mean half-life around 30 hours), mTOR inhibitors (mean half-life around 30 hours) at least for the last 7 days prior to T-cell infusion, and radiotherapy or major surgery within the last 2 weeks prior to T-cell infusion. For PD-1/PD-L1 inhibitors or CTLA-4 inhibitors, a 4-week period must have elapsed before T-cell infusion. For recent experimental therapies a 28-day period must have elapsed before infusing expanded T-cells.
- Patients with active CNS involvement by malignancy either by imaging or cerebrospinal fluid involvement or biopsy-proven (due to poor prognosis and potential for neurological dysfunction that would confound evaluation of neurological and other adverse events) **except for:**

a) Patients with 3 or fewer brain metastases of <1cm treated with either stereotactic or gamma knife radiotherapy and remained stable on MRI for 2 weeks are eligible.

b) Patients with surgically resected brain metastases and no evidence of active disease in the CNS at the time of screening evaluation are eligible.

- Patients with hypercalcemia (>10 mg/dL) of malignancy.
- Any prior Grade ≥ 3 immune-related adverse event (irAE) while receiving immunotherapy, including anti-CTLA4 treatment that requires long-term immunosuppressive therapy. Note: Active or history of vitiligo or hypothyroidism will not be a basis for exclusion.

- Patients with second malignancies in addition to their clear cell RCC are not eligible if the second malignancy has required systemic treatment within the past 4 years or is not in complete remission. There are exceptions to this criterion: successfully treated non-metastatic basal cell, squamous cell skin carcinoma, in situ non-invasive cervical cancer and in situ non-invasive breast cancer.
- Women of child-bearing potential who are pregnant or breastfeeding because of the potentially dangerous effects of the preparative chemotherapy on the fetus or infant.
- Active coagulation disorders or other major uncontrolled medical illnesses of the respiratory, endocrine, renal, gastrointestinal, genitourinary or immune system, uncontrolled systemic infection, active obstructive or restrictive pulmonary disease.
- Patients who have recent history of cerebrovascular accident, transient ischemic attack should be cleared by the neurology consult service before enrolling this study.
- Patients who have recent history of coronary artery disease or cardiac arrhythmia should be cleared by the cardiology consult service before enrolling this study.
- Any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease).
- Patients with autoimmune diseases such as Crohn's disease, ulcerative colitis, rheumatoid arthritis, autoimmune hepatitis or pancreatitis, and systemic lupus erythematosus that requires treatment with chronic immunosuppressive therapy.
- Systemic corticosteroid steroid therapy of any dose is not allowed within 2 days prior to enrollment. The following are exceptions to this criterion:
  - Intranasal, inhaled, and topical steroids
  - Systemic corticosteroids at physiologic doses not to exceed 10 mg/day of prednisone or equivalent
  - Steroids as premedication for hypersensitivity reactions (e.g., CT scan premedication).
- History of severe immediate hypersensitivity reaction to any of the agents used in this study.
- Unable to understand the investigational nature of the study or give informed consent and does not have a legally authorized representative or surrogate that can provide informed consent per section 14.
- Any condition that, in the opinion of the investigator, would interfere with evaluation of the investigational product or interpretation of subject safety or study results.

5. CLINICAL EVALUATION OF THE PARTICIPANT

Study eligibility is based on meeting all of the study inclusion criteria and none of the exclusion criteria at screening before study treatment administration. Screening evaluations will be performed as part of an NIH Screening protocol. Laboratory results from other NIH IRB approved protocols that subjects may have participated in within 30 days of enrollment in this study may also be used as part of the pre-study evaluation.

Protocol evaluation (on 97-H-0041)

- Complete history and physical examination, including, weight, height, vital signs and ECOG performance status.
  - Patients with favorable, intermediate and poor risk categories will be eligible for the study. We will include poor prognostic factors in the baseline H&P the following as described per International Metastatic RCC Database Consortium (IMDC):
    - KPS equal to 70 or less
    - Less than 1 year from diagnosis to initial systemic treatment
    - Hemoglobin less than the LLN
    - Corrected calcium concentration greater than 10 mg/dL
    - Absolute neutrophil count greater than the ULN
    - Platelet count greater than the ULN

- ECG
- Confirmation of diagnosis of a clear cell renal cell carcinoma by the NIH Laboratory of Pathology. The sample used for the confirmation of the diagnosis can come from any time prior to enrollment on the protocol. The sample can be a fresh biopsy or paraffin-fixed slides.
- 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, AST, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid.
- CBC with differential.
- Pre-albumin and lipid panel
- PT/PTT.
- TBNK studies
- High resolution HLA typing to confirm HLA-A* 11:01 typing.
- Antibody screen for Hepatitis B, Hepatitis C and HIV.
- Anti-HTLV-I/II, Anti-HBcAntibody, West Nile Virus, HIV-1/HCV/HBV NAT, T. Cruzi Antibody, Toxoplasma Antibody. Syphilis (RPR testing) (DTM blood screening panel)
- Anti-CMV antibody titer, HSV serology and EVB panel.
- Chest radiograph (Posterior-Anterior and Lateral views).
- CT of the neck with IV contrast in patients with known or suspected metastatic disease in the neck (or MRI of the neck with IV gadolinium contrast if clinically indicated)
- CT with IV contrast of the chest/abdomen/pelvis (or CT chest without contrast plus MRI abdomen/pelvis with IV gadolinium contrast if clinically indicated) to document measurable malignancy.
  Note: studies are to be performed with contrast unless renal impairment or allergy prohibits the use of contrast.
- Tc99-bone scintography (and/or whole-body MRI if clinically indicated) in patients with known or suspected bone metastases.
- MRI of the brain with IV gadolinium contrast (or CT of the brain with IV contrast if indicated) **Note:** Studies are to be performed with contrast unless renal impairment or allergy prohibits the use of contrast.
- Cardiac function: echocardiogram
- Pulmonary function tests.
- β-HCG pregnancy test (serum or urine) on all women of child-bearing potential.
- Urine analysis/random Urine Protein-Creatinine Ratio or 24-hour urine collection if clinically indicated.
- Type and screen + DAT
- Venous access assessment. Placement of peripheral IV catheter *versus* Central Venous Catheter Placement (when indicated) will be performed by the NIH Procedures, Vascular Access and Conscious Sedation (PVCS) Service. A separate consent will be obtained at the time of the procedure.
- Research blood for enumeration of absolute # of circulating CD34+/CD8+ T-cells [30 ml of blood collected in a green tube] will be collected in patients that are screened for re-treatment with HERV-E TCR transduced CD8+/CD34+ enriched T-cells and a second apheresis is needed.

Baseline evaluation and procedures under this protocol (within 8 weeks of initiating chemotherapy)
The following assessments must be completed within 4 weeks prior to starting the leukapheresis and/or 8 weeks of initiating chemotherapy unless otherwise noted (if not, then the evaluation must be repeated). Any of these procedures done during screening do not need to be repeated for baseline analysis.

- Interim history and physical examination, including, weight, height, vital signs and ECOG performance status. Note in detail the exact size and location of any lesions that exist.

- Sodium, Potassium, Chloride, Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total, Inorganic Phosphorus, Alkaline Phosphatase, ALT, AST, Total Bilirubin, Direct Bilirubin, LDH, Total Protein.
- CBC with differential.
- PT/PTT and D-dimer
- TBNK studies
- C-reactive protein
- Urine analysis and random Urine Protein-Creatinine Ratio (or 24-hour urine collection if clinically indicated)
- Glucose-6- phosphate dehydrogenase (G6PD) deficiency screening.
- Thyroid panel (TSH, if TSH is abnormal, then T4).
- Serum cortisol
- β-HCG pregnancy test (serum or urine) on all women of child-bearing potential.
- RCR testing
- Research blood as per Table 8 and SOE
- Durable Power of Attorney Form Completed
- F18-FDG-PET CT –obtained **within 2 weeks** of initiating chemotherapy as a baseline procedure
- CT of the neck with IV contrast in patients with known or suspected metastatic disease in the neck. (or MRI of the neck with IV gadolinium contrast if clinically indicated)
- CT with IV contrast of the chest/abdomen/pelvis (or CT chest without contrast plus MRI abdomen/pelvis with IV gadolinium contrast if clinically indicated) to document measurable malignancy will be obtained within 2 weeks of initiating chemotherapy as a baseline procedure. Note: studies are to be performed with contrast unless renal impairment or allergy prohibits the use of contrast.
- Imaging tests will be obtained within 2 weeks of initiating chemotherapy as a baseline procedure. Note: studies are to be performed with contrast unless renal impairment or allergy prohibits the use of contrast.

Optional Biopsies or Malignant Effusions

Selection of lesion amenable for biopsy and target lesions in consultation with radiology within 4 weeks after leukapheresis but prior to conditioning chemotherapy. Tumor biopsies (nodal or visceral) will be optional for analyses of HERV-E expression, tumor line establishment, VHL mutation status and other molecular analysis and will be obtained at baseline and may be obtained at different time points after the HERV-E TCR transduced CD8+/CD34+ enriched T-cells infusion and at the time of disease progression. If patient happens to have a malignant effusion, we may request a sample of the effusion via pleural aspiration or paracentesis for research purposes such as tumor line establishment.

During the preparative chemotherapy regimen: (mandatory only D-4 prior to chemotherapy unless noted)

- Daily vital signs **(Daily)**
- Weight
- Complete Blood Count with differential.
- Sodium, Potassium, Chloride, Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total, Inorganic Phosphorus, Alkaline Phosphatase, ALT, AST, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid
- C-reactive protein
- Research blood (on days specified in Table 8).

Immediately after Cell Infusion:

- Vital signs will be monitored hourly (+/- 15 minutes) until stable and then routinely (every 4-6 hours) unless otherwise clinically indicated.

Studies to be performed during inpatient admission after cell infusion

- Daily interim history and physical examination, including, weight, and vital signs
- Vital signs including pulse oximetry will be monitored every 4-6 hours unless otherwise clinically indicated.
- CBC once daily until discharge
- TBNK studies on the day of HERV-E TCR transduced T-cells infusion on day 0 (cell infusion) and every other day through day 6. On D0, D+2, D+4 and D+6
- Sodium, Potassium, Chloride, Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total, Inorganic Phosphorus, Alkaline Phosphatase, ALT, AST, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid, once daily until discharge.
- PT/PTT once daily until discharge.
- C-reactive peptide on D0, D+2, D+4 and D+7
- Thyroid panel (TSH, if TSH is abnormal, thenT4) and antithyroid panel (THY2) only on Day 0 and D+7
- Type and screen every 4 days
- Research blood (as specified in Table 8).

Post-infusion outpatient evaluation

Patients will be seen at the NIH (OP7) in follow-up to evaluate disease status and late problems related to HERV-E TCR transduced T-cells infusion at the following time-points: after hospital discharge patient may be seen twice a week (+/- 2 days) for clinical and safety evaluation up to day +14 (+/- 2 days) , then on days +21 (+/- 3 days), +30 (+/- 5 days), +60 (+/- 7 days), +90 (+/- 7 days), +120 (+/- 14 days), +180 (+/- 14 days), +240(+/- 14 days), +300 (+/- 30 days) and +360 (+/- 30 days) after HERV-E TCR transduced $CD8^+/CD34^+$ enriched T-cells infusion and when clinically indicated.

At all outpatient follow-up visits unless otherwise noted, patients will have the following tests performed to determine clinical response and toxicities. Health status data will be obtained from surviving patients for a total of 15 years after cell infusion.

- Interim history and physical examination/SOAP note, including, weight, vital signs and ECOG performance status.
- CBC with differential.
- Sodium, Potassium, Chloride, Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total, Inorganic Phosphorus, Alkaline Phosphatase, ALT, AST, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid.
- PT/PTT.
- C-reactive peptide
- TBNK cell analysis on day +14 and then every follow-up appointment post HERV-E TCR transduced T-cells infusion.
- Blood for serum quantitative immunoglobulins on day +21 and then only as clinically indicated.

- Thyroid panel (TSH, if TSH is abnormal, thenT4) and antithyroid panel (THY2) on Day+14 and then only if prior abnormal as dictated by PI.
- CT scans of the neck (if applicable based on baseline disease), chest, abdomen, and pelvis with IV contrast (or CT scan of the chest without contrast and MRI of the neck, abdomen and pelvis with IV gadolinium contrast if clinically indicated) at outpatient follow-up appointments starting day +30 after infusion and then as indicated in SOE (D+90, D+120, D+180, D+240, D+300 and D+360). CT scans will not be obtained at D+60 unless tumor assessment at D+30 is consistent with disease progression. Note: studies are to be performed with contrast unless renal impairment or allergy prohibits the use of contrast.
- Tc99-bone scintigraphy and/or whole-body MRI will be obtained only in those patients with known bone metastasis stating on day +30 after infusion and then as indicated in SOE. (D+90, D+120, D+180, D+240, D+300 and D+360). Tc99-bone scintigraphy and/or whole-body MRI will not be obtained at D+60 unless tumor assessment at D+30 is consistent with disease progression.
- MRI of the brain with IV gadolinium will be obtained only in those patients with known brain metastasis starting on day +30 after infusion and then as indicated in SOE (D+90, D+120, D+180, D+240, D+300 and D+360) or when clinically indicated. MRI of the brain will not be obtained at D+60 unless tumor assessment at D+30 is consistent with disease progression
- All patients should get a F18-FDG-PET CT scan at day +60, +120, +180, +240, +300 and +360 follow-up visits and anytime a F18-FDG-PET CT is clinically indicated.
- MRI for specific body area if metastasis present will be performed only if clinically indicated
- Research blood for correlative studies as described in SOE and Table 8.
- Detection of RCR and persistence of TCR gene transduced cells: (3mo, 6mo, 1yr. see Follow-up SOE).
- Long-term follow up of patients receiving gene transfer: Patients will be followed as described below. Physical examinations will be performed and documented annually at least for 5 years following cell infusion to evaluate long-term safety. After 5 years, health status data will be obtained from surviving patients via telephone contact or mailed questionnaires. The long-term follow up period for retroviral vectors is 15 years.

NOTE: All CT scan with IV contrast of the abdomen and pelvis must be ordered with artherial phase. Other tests will be performed as clinically indicated.

Subjects will begin long term follow up one year following the last gene therapy administration, or at the time of disease progression (if occurring <12 months after gene therapy), as described below:

- **Years 1 -5:** Annual history and physical examination from a health care provider. It is preferable but not necessary for this to be performed at the Clinical Center. If the patient elects not to return, a copy of the physical exam should be obtained from the patient's referring health care provider and the health status information should be obtained via phone or e-mail with the patient.

- **Years 6 -15 (if required):** Annual health status check via telephone or e-mail contact with the patient.

- Patients who are treated with gene therapy which includes a retroviral vector (including gamma-retroviral vectors and lentiviral vectors), will be requested to send a blood sample to the NIH at 3, 6, and 12 months post-treatment, and annually thereafter for 4 more years. These blood samples will be used along with a pre-treatment sample to assay for replication-competent retroviruses. If any indication of gene therapy-related toxicity develops, additional blood samples will be required to define and investigate the toxicity.

- Patients who are treated with gene therapy and persistence of HERV-E TCR transduced $CD8^+/CD34^+$ T-cells in peripheral blood at the 12-month time-point will be requested to send a blood sample (Table 8) to NIH every 6 months up to 5 years and then yearly up to 15 years to 1 year for:
  - Quantification of HERV-E TCR transduced $CD8^+/CD34^+$ enriched T cells.
  - Cytolytic potency/activity of HERV-E TCR transduced $CD8^+/CD34^+$ enriched T cells.
  - HERV-E TCR transduced $CD8^+/CD34^+$ enriched T cell surface phenotype and immune subsets.
  - Comprehensive cytokine/Chemokine studies.

Please Note: Patients who are unable or unwilling to return for follow up evaluations will be followed via phone or e-mail contacts. A request will be made to send laboratory, imaging and physician exam reports performed by their treating physician; and any outstanding toxicities will be reviewed with the patient.

6. TREATMENT PLAN

Drug Administration

- **Leukapheresis**

The patient will undergo a 12-15 (+/- 500 ml) liters leukapheresis-in the DTM Dowling Apheresis Clinic according to DTM standard operating procedures. The procedure requires dual venous access and takes approximately 3-4 hours to complete. A central line will be placed if peripheral venous access is not sufficient. The apheresis product will be processed to purify the PBMC, transduced to express the HERV-E TCR, and prepared for patient infusion.

NOTE: Dr. Nishimura's team may retain excess PBMCs from initial apheresis collection. PBMCs will be cryopreserved under GMP conditions and stored at Loyola University or another ORSC-approved contracted manufacturing facility. Frozen PBMCs may be used to manufacture CD8+ T cells transduced with HERV-E TCR.

- **Preparation of the HERV-E T cell receptor transduced T cells for adoptive cell transfer**

After cells are obtained by apheresis, they will be shipped to LUC or another ORSC-approved manufacturing facility for processing and TCR transduction per SOPs. Freshly-collected cells will be used to initiate the cell-preparation process as described in section 3.2. HERV-E TCR- expressing T cells will be cryopreserved at LUC or another ORSC-approved contracted manufacturing facility on day 21 and shipped to NIH. Cells will be released for infusion after 7 days of negative cultures are confirmed on the day 21 expanded T-cell product.

- **Conditioning therapy and autologous HERV-E TCR transduced T-cell administration.**

All patients will receive a non-myeloablative lymphocyte depleting preparative regimen of fludarabine (30 mg/m^2/day IV) on days -4 through -2 and cyclophosphamide (1,000 mg/m^2/day IV) on day -4 (Table 6). The non-myeloablative lymphocyte depleting preparative regimen can be administered as an inpatient or outpatient. Patients will be admitted to NIH inpatient ward on day -1, if not before, for administration of HERV-E TCR transduced T-cells (Table 6).

The day of the cell infusion is day 0. It will be a single infusion according to the CC nursing standards of practice guidelines. During cell infusions, the subjects will be evaluated for side effects and possible toxicities of the infusion, which include flu-like symptoms. Patients will remain on the inpatient service

as needed (at a minimum days 0 through 7). Patients will start moderate -dose IL-2 2,000,000 IU/m^2 given every 12 hours by intravenous infusion for 14 doses beginning as soon as practical after completing the cell infusion (Table 6).

- **Line Placement**

Subjects will have inserted a midline catheter, a peripherally inserted central catheter (PICC) or another type of central venous catheter placed prior to chemotherapy administration to aid with blood draws and ensure infusion of the expanded T-cells and IL-2 administration.

Table 6. Overall summary of the treatment plan

Day	-4	-3	-2	-1	0	1	2	3	4	5	6	7
Therapy												
Cyclophosphamide IV 1000 mg/m²	X											
Fludarabine IV 30 mg/m²	X	X	X									
Infusion of HERV-E TCR transduced CD8⁺/CD34⁺ T-cells					X							
IL-2 IV 2,000,000 IU/m² q12h for 14 doses					X	X	X	X	X	X	X	X

Concomitant Medications/Measures

INFECTION PROPHYLAXIS

Note: Appropriate anti-infective agents may be substituted at the discretion of the treating physician.

- **Pneumocystis Jirovecii Pneumonia**

All patients will receive the fixed combination of trimethoprim and sulfamethoxazole (TMP/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 on day -4. 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 prior to admission and monthly thereafter. PCP prophylaxis will be stopped after 2 consecutive CD4 counts greater than 200/µL are documented, first tested at least 30 days following the conditioning regimen or when clinically indicated

- **Herpes Virus Prophylaxis**

Patients will be given acyclovir orally at a dose of 800 mg twice daily, beginning on day -4, or acyclovir, 250 mg/m2 IV every 12 hours if the patient is not able to take medication by mouth. Reversible renal insufficiency has been reported with IV but not oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs have been reported with higher doses of acyclovir. Should this occur, a dosage adjustment will be made, or the drug will be discontinued. Acyclovir will not be used concomitantly with other nucleoside analogs which interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling. Prophylaxis for Herpes will continue until the CD4 count is greater than 200 for 2 consecutive measures or when clinically indicated

- **Fungal Prophylaxis**

Primary antifungal prophylaxis for cryptococcal disease is not mandated. Patients will start Fluconazole 200 mg p.o. starting on the day of cell infusion and continue until clinically indicated only at the PI

discretion. The drug may be given IV at the same dose in 0.9% sodium chloride USP daily in patients unable to take it orally.

- **Neutropenia**

A CBC with differential will be obtained daily during inpatient admission. **If the absolute neutrophil count becomes less than 500/microliter, filgrastim or filgrastim-sndz may be initiated** at a dose of 300 micrograms daily subcutaneously for patients under 70 kg in weight and at a dose of 480 micrograms daily for patients 70 kg and over in weight. Filgrastim or filgrastim-sndz will be given daily until absolute neutrophil count recovers to > 1500/µL.

- **Empiric Antibiotics**

Patients will start on broad-spectrum antibiotics, in accordance with current institutional guidelines for fever of 38.3°C once or two temperatures of 38.0°C or above at least one hour apart, AND an ANC <500/mm3. Infectious disease consultation will be obtained for all patients with unexplained fever or any infectious complications.

Table 7. Prophylactic Treatments

Prophylactic treatment	Dosage	Administration
TMP/SMX - DS	160mg/800mg PO daily	Every Monday-Wednesday-Friday beginning on day -4. PCP prophylaxis will be stopped after 2 consecutive CD4 counts greater than 200/µL are documented or when clinically indicated
Pentamidine* (Pentamidine will be substituted for TMP/SM in patients with sulfa alle	Aerosolized at 300 mg per nebulizer	PCP prophylaxis will be started within one week of chemotherapy start date and then monthly. PCP prophylaxis will be stopped after 2 consecutive CD4 counts greater than 200/µL are documented or when clinically indicated
Fluconazole	200 mg PO daily If unable to tolerate PO: Fluconazole 100 mg in 0.9% sodium chloride USP daily IV daily	Primary untifungal prophylaxis for cryptococcal disease is not mandated. Antifungal Prophylaxis will start on day 0 and will be administered only at the discretion of the PI and until clinically indicated.
Acyclovir	800mg PO twice daily (preferred) If unable to tolerate PO: acyclovir 250mg/m2 IV every 12 hour	HSV and VZV prophylaxis will be started days -4 and stopped after 2 consecutive CD4 counts greater than 200/µL are documented or when clinically indicated
Filgrastim	300 micrograms daily for patients under 70 kg in weight and a dose of 480 micrograms daily for	Until absolute neutrophil count recovers to > 1500/µL

	patients over 70 kg in weight may be started as per PI discretion	

Detailed Treatment Plan

Conditioning chemotherapy and HERV-E TCR transduced T-cells -this can be either inpatient or outpatient
Note: No subjects will be treated at Loyola University.

Day -4, -3, and -2: Patients will receive pre-hydration with 1000 mL 0.9% sodium chloride I.V. over 1 to 3 hours on day -4. Patients will receive anti-emetics following NIH Clinical Center guidelines, but **dexamethasone will not be administered**. One suggested regimen is ondansetron 16 to 24 mg orally on days -4, -3, and -2 one hour before chemotherapy (I.V. ondansetron can be substituted). Patients will be provided with anti-emetics such as lorazepam and prochlorperazine to use at home.
On day -4, cyclophosphamide at a dose of 1000 mg/m^2 I.V. will be diluted in 100 ml 5% dextrose solution and infused. After the cyclophosphamide on day -4, patients will receive 30 mg/m^2 I.V. fludarabine in 100 mL 0.9% sodium chloride. Following the fludarabine infusion, patients will receive 1000 mL 0.9% sodium chloride I.V. over 1-2 hours. Furosemide will be given if needed.

Next, on days -3, and -2, patients will receive 30 mg/m^2 I.V. fludarabine in 100 mL 0.9% sodium chloride.

Note: in patients with a creatinine clearance calculated by the CKD-EPI equation less than 80 ml/minute/1.73 m^2 of body surface are, the daily dose of fludarabine will be reduced by 20%.

Note: The dose of fludarabine can be rounded to the nearest vial size to conserve medication as long as the dose reduction is ≤10% of the calculated dose.

Day -1: No interventions except as needed for general supportive care such as anti-emetics. To minimize bladder toxicity, patients should increase normal oral fluid intake to at least 2 liters/day.

Day 0: HERV-E TCR transduced $CD8^+/CD34^+$ enriched T cells will be thawed in the DTM and following confirmation that they meet release criteria, will be administered to the patient. The pre-medications are acetaminophen 650 mg orally and diphenhydramine 25mg IV. Cells will be delivered to the patient care unit from DTM. Prior to infusion, the cell product identity label is double-checked by two authorized staff (MD or RN), and 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 according to the CC nursing standards of practice guidelines.

Days 0 to 7: Mandatory hospitalization for observation and treatment as necessary. IL-2 2,000,000 IU/m^2 given every 12 hours IV infusion for 14 doses beginning as soon as practical after completing the cell infusion. One dose of IL-2 may be started on Day 0, and then every 12 hours for a total of 14 doses. Doses will be preferentially administered every twelve hours; however, up to 24 may elapse between doses depending on patient tolerance. Dosing time adjustment may be performed at the investigator's discretion.

6.4 Blood Product Support

- Leukocyte filters will be utilized for all blood and platelet transfusions except for the HERV-E TCR transduced T-cells to decrease sensitization to transfused WBC and decrease the risk of CMV infection.
- 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 Hgb >8.0 gm/dl, and platelets >10,000/μL.
- All blood products except for the HERV-E TCR transduced T-cells will be irradiated.

6.5 Anti-Emetics

Anti-emetics Guidelines (except that corticosteroids will be avoided).

6.6 Granulocyte Colony-Stimulating Factor

If the absolute neutrophil count becomes less than 500/microliter, filgrastim or filgrastim-sndz may be initiated at a dose of 300 micrograms daily for patients under 70 kg in weight and a dose of 480 micrograms daily for patients over 70 kg in weight. Filgrastim or filgrastim-sndz may be discontinued as soon as the absolute neutrophil count recovers to 1500/μL.

6.7 Avoidance of Corticosteroids

Patients should not take systemic corticosteroids including prednisone, dexamethasone or any other corticosteroid at any dose for any purpose without approval of the Principal Investigator.

RE-TREATMENT

Patients experiencing a partial or complete response or stable disease for at least 3 months (+/- 1 week) (SD measured at the tumor assessment on D+90 associated with relief of one or more tumor-associated symptoms) may receive one additional retreatment at a higher dose level (i.e. a higher cell dose) when progression by RECIST criteria is documented after evaluation by the Principal Investigator. Such patients would be retreated at the currently enrolling dose level, which must be a higher dose level than the initial treatment. Patients retreated at a higher dose level will be counted for the primary study endpoint in both dose levels to determine the maximum tolerated dose, as the use of conditioning lymphodepletion chemotherapy given before the second T-cell infusion should remove any remaining HERV-E TCR transduced T cells from circulation or tissues, and patients will be only enrolled if there is no evidence for any active immune-mediate toxicities related to prior treatment.

Available frozen apheresis cells used for the initial TCR transduction that were collected prior to the first T cell infusion will be the preferred cell source for manufacturing the 2nd cell product to be used at the higher dose level.

The collection of a fresh apheresis product can be used to manufacture the 2nd cell product when a prior frozen apheresis sample is not available for further T-cell transduction. Fresh 2nd apheresis product will not be collected, if <u>any</u> circulating CD34^{+}/CD8^{+} T-cells (these are the transduced T-cells) are detectable in the circulation from the first infusion. This will exclude any possibility of toxicity being caused by any T-cells in the 2nd collection that were remaining transduced T-cells from the original transduction.

Any patients who developed a grade 3 or grade 4 toxicity possibly or definitely related to the HERV-E TCR T-cell product will not be eligible for retreatment at a higher dose level. Patients must continue to meet the

original eligibility criteria to be considered for retreatment. Retreatment benefits and risks will be carefully explained to the patient. A maximum of 1 retreatment course may occur.

7. MANAGEMENT OF COMPLICATIONS

7.1 Dose Modification/Delay

For patients who have yet to receive treatment, patients may be removed from treatment or have their treatment delayed if they have active infections defined as infections causing fevers or infections requiring intravenous anti-microbial therapy and intravenous anti-microbial therapy occurring less than 72 hours before the start of chemotherapy. This means that patients are on-study but have not yet started treatment could have treatment cancelled or delayed; but, such patients are eligible for treatment after the infection resolves. If a patient experiences a grade 3 or greater toxicity (except for cytopenias or nausea or other toxicities as per investigator's discretion) while on-study before the HERV-E TCR transduced T-cells infusion, the HERV-E TCR transduced CD8+/CD34+ enriched T-cells infusion must be delayed until the toxicity improves to a grade 2 or less or cancelled.

7.2 Cytokine release syndrome

Cytokine release syndrome (CRS), which will be graded according to the CRS grading scale of Lee *et al*.[141] Please see section Appendix C. These are guidelines only. It is understood that treatment of these toxicities must be individualized for each patient. Not following the exact recommendations in Appendix C is not a protocol deviation.

7.3 IL-2 Toxicities

Guidelines for dealing with toxicities that often occur after HERV-E TCR- expressing T cells infusions and IL-2 treatment are given in Appendix D. In addition, patients are required to stay within 60 minutes driving distance from the Clinical Center until day 30 (+/- 7 days) after the HERV-E TCR-expressing T cells infusion.

8. BIOSPECIMEN COLLECTION

8.1 Tumor Tissue

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

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. Samples will be ordered and tracked through the CRIS Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record. Samples will not be sent outside NIH without IRB notification and an executed MTA.

Archival Tissue

Archival tissue (Recommended: 15 unstained slides representative of the tumor for diagnosis confirmation and 15 unstained slides or 1 tumor block to be used for research purposes), will be required for enrollment

onto the study for diagnosis confirmation. The patient will have the option to undergo a pre- treatment biopsy if archival tissue is not available for diagnosis confirmation. (preferably of metastatic disease).

Fresh Tissue and Malignant Effusions
Optional tumor biopsies or sampling of malignant effusions as described below are obtained before the initiation of study therapy, on treatment and at the time of progression (all of them are optional tumor samples). In the case of autopsy, we may request samples from different organs and tumor for research purposes.

Fresh tissue samples should be collected into empty, unused barcoded cryovials (Nunc catalog #5001-0012, 5001-1020 or equivalent) and stored on dry ice or liquid nitrogen. Tissue may be obtained by core biopsy, surgical dissection, or aspiration in the case of malignant effusions. The interval between sample acquisition and freezing on dry ice must not exceed 5 minutes. Frozen, vialed samples should be transferred from dry ice to -80°C (minimum) or liquid nitrogen (preferred) frozen storage within 2-3 hours.

The tumor tissue will be divided among two laboratories:

- Laboratory of pathology (Maria Merino or designee) will perform diagnosis confirmation (Recommended: 15 unstained slides representative of the tumor).
- Laboratory of Transplantation Immunotherapy (Recommended: 15 unstained slides or 1 tumor block, fresh tissue, and malignant effusions). The recommended slides received will be first evaluated by Dr. Merino or designee for pathology assessment and then stored at the Laboratory of Transplantation Immunotherapy.

- **Samples sent to the Laboratory of Transplantation Immunotherapy, Dr. Childs' Lab**

Venous blood samples will be collected to be processed for serum if appropriate and stored for future research. Record the date and exact time of draw on the tube. Tissue will be processed as described and stored for future research.

- For questions regarding sample processing or for immediate help, contact Robert Reger by email (robert.reger@nih.gov) or at 301.594.8004, or Elena Cherkasova by email (cherkasovae@nhlbi.nih.gov) or at 301-827-3958.
- The samples will be processed, barcoded, and stored in Dr. Childs's lab until used.

8.2 Immunological Testing (Exploratory studies)

- Samples of all infused cell products will be cryopreserved, and extensive retrospective analysis of infused cell phenotype and function will be performed to attempt to find *in vitro* characteristics of the infused cells which correlate with *in vivo* antitumor activity. Analyses of peripheral blood lymphocytes (PBL) samples will include evaluation of the activity and specificity of infused CD8+/CD34+ enriched T cells.
- Lymphocytes will be tested directly and following *in vitro* culture using some or all of the following tests. Direct immunological monitoring will consist of quantifying T cells reactive with targets FACS analysis using mouse V-beta antibody. *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 transduced PBMC cryopreserved at the time of freezing the product. In general, differences of 2 to 3 fold in these assays are indicative of true biologic differences.

- Research apheresis may be performed after the treatment. Apheresis product will be transferred to Dr. Childs' lab, Section of Transplantation Immunotherapy, attention Robert Reger (robert.reger@nih.gov), building 10/CRC, room 5-5264. Possible laboratory research studies on PBMC and PBL are as follows: Specific cytolysis determined by calcein AM assay, quantity of cytokine production as determined by coculture assay with cytokine quantification, cytokine production by intracellular flow cytometry, phenotypic analysis by flow cytometry.

8.3 Monitoring Gene Therapy Trials: Persistence and Replication Competent Retrovirus (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.
- Patients' blood samples will be obtained and undergo analysis for detection of RCR by PCR prior to cell infusion approximately at 3 and 6 months, and at one-year (+/- 30 days) 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 or after this trial, efforts will be made to assay a biopsy sample for RCR. If any post-treatment samples are positive, further analysis of the RCR and more extensive patient follow-up will be undertaken, in consultation with the FDA. RCR PCR assays detect the GaLV envelop gene and are performed under contract by the Indiana University Vector Production Facility. The results of these tests are maintained by the contractor performing the RCR tests and by the NHLBI research team.

  **NOTE: We will obtain 1 EDTA tube with 4-5 ml of peripheral blood. These samples need to be sent on ice packs priority overnight to the attention of:
  Lisa Duffy
  IU Vector Production Facility
  980 W. Walnut St.
  R3-C668
  Indianapolis, IN 46202

  RCR testing sample will need to be shipped the day drawn if at all possible. IU Vector Production Facility is not open on the weekends, if a sample must be drawn on a Friday, it will be placed at 4ºC over the weekend and shipped on Monday.

8.4 Post Infusion Blood Samples and Optional Tumor Tissue for Exploratory Studies

Correlatives studies including peripheral blood (mandatory) and fresh tumor tissue or malignant effusions (recommended but optional) will be collected at baseline, on-treatment (pre-determined time points) and at the time of progressive disease. If available, samples collected under protocol 04-H-0012 can be used for the research purposes of this protocol as described below.

- **CT-RCC HERV-E expression.** Determine target tumor-associated antigen expression levels in tumor tissues (fresh and/or FFPE-fixed). This will be done by qRT-PCR of total RNA extracted from tumor material using CT-RCC HERV-E specific primers.
- **Quantification of HERV-E TCR transduced $CD8^+/CD34^+$ enriched T cells.** Evaluate the *in vivo* persistence and peak blood levels of HERV-E TCR transduced $CD8^+/CD34^+$ T cells. This will be measured by staining with anti-CD8 and anti-CD34 antibodies. The methods for immunofluorescence staining of T cells have been published.[142]
- **Cytolytic potency/activity of HERV-E TCR transduced $CD8^+/CD34^+$ enriched T cells.** *Ex vivo* immunological assays will be used to measure the antigen-specific functional activity of the HERV-E TCR transduced $CD8^+/CD34^+$ enriched T cells and will consist of intracellular cytokine staining, chemokines release assays and cytotoxicity assays. Immunological assays will be standardized by the inclusion of pre-infusion recipient PBMC and in some cases an aliquot of the engineered T cells cryopreserved at the time of infusion.
- **HERV-E TCR transduced $CD8^+/CD34^+$ enriched T cell surface phenotype and immune subsets.** T cell subset analysis will be performed to evaluate markers of T cell activation and exhaustion in HERV-E TCR transduced $CD8^+/CD34^+$ enriched T cell over time and to measure lymphoid reconstitution.
- **Comprehensive cytokine/Chemokine studies.** Multiple cytokines and chemokines are necessary for effective host immunity against tumor cells. Correlate these chemical signals that attract T cells to infiltrate the tumor and activate targeted cytotoxic responses with presence of HERV-E TCR transduced $CD8^+/CD34^+$ enriched T cells in tumor and response.
- **Presence of HERV-E TCR transduced $CD8^+/CD34^+$ enriched T cells in tumor tissue.** Determine the infiltration of HERV-E TCR transduced $CD8^+/CD34^+$ enriched T cells in tumor tissue.
- **Tumor Immune Subsets.** Determine recruitment and activation of immune effector cells in tumor tissue.
- **Establishment of cell lines** from patient tumor cell biopsies if possible.

Table 8. Summary of biospecimen collection, handling, distribution and storage

Test Description	Amount/Type/ Tube/Handling	Collection Timing	Contact	Special Instruction
Diagnostic Confirmation	Recommended 15 unstained slides representative of the tumor	Baseline pre-treatment	Kristen Gunn Email: kristen.gunn@nih.gov Phone #: 301-827-2977	Tissue will be submitted to Pathologist .
HERV-E expression in paraffin-embedded tumors	Recommended 15 unstained slides or 1 block of tumor	Baseline pre-treatment	Kristen Gunn Email: kristen.gunn@nih.gov Phone #: 301-827-2977	Tissue will be submitted to Pathologist for initial evaluation and then stored at the Laboratory of Transplantation Immunotherapy.
Baseline pre-treatment: - Immune subsets characterization	Optional Fresh Tumor Biopsy (1-5) or malignant effusions at NIH	Baseline pre-treatment, on treatment and at the time of disease	Email: Robert Reger by e-mail (robert.reger@nih.gov) or at 301.594.8004, 24 hours prior to anticipated collection	1-5 cores will be flash frozen at the time of biopsy and malignant effusion in a vacuum bottle.

-Generate tumor cell lines **On-Treatment:** - Presence of HERV-E TCR transduced $CD8^+/CD34^+$ T-cells and generate cell cultures **Disease Progression:** -Presence of HERV-E TCR transduced $CD8^+/CD34^+$ T-cells and generate cell cultures		progression or other time points if clinically indicated. i.e. drainage of malignant effusions	Then contact Robert Reger by e-mail (robert.reger@nih.gov) or at 301.594.8004, or Elena Cherkasova by email (cherkasovae@nhlbi.nih.gov) or at 301-827-3958 for questions with regards sample collections instructions and immediate pick-up.	When the patient is scheduled for biopsy will call Robert Reger (301.594.8004) and cores will be distributed accordingly. 1-5 cores will be sent to Dr. Child's laboratory for further processing and experiments.
Enumeration of absolute number of circulating HERV-E TCR transduced $CD8^+/CD34^+$ T-cells by Flow cytometry*	30 ml of peripheral blood collected in green top tubes	D-4, D0, D+2, D+4 and D+7 after T cell infusion and then on day +14, and then every planned follow-up appointment	Email: Robert Reger, robert.reger@nih.gov 24 hours prior to anticipated collection Please contact Robert Reger by e-mail (robert.reger@nih.gov) or at 301.594.8004, or at 240-750-4660 or Elena Cherkasova by email (cherkasovae@nhlbi.nih.gov) or at 301-827-3958 for questions about sample collections instructions.	After the tube has been filled with blood, immediately invert the tube several times to prevent coagulation.
Interferon-γ release and cytotoxicity assays*		D-4, D0, D+2, D+4 and D+7 after T cell infusion and then on day +14 and then every planned follow-up appointment	-	-
Intracellular cytokine staining of HERV-E TCR transduced $CD8^+/CD34^+$ T-cell*		D-4, D0, D+2, D+4 and D+7 after T cell infusion and then on day +14 and then every planned follow-up appointment	-	-

HERV-E TCR transduced $CD8^+/CD34^+$ T-cell surface phenotype and activation status*		D-4 , D0, D+2, D+4 and D+7 after T cell infusion and then on day +14 and then every planned follow-up appointment	-	-
Cytokine/chemokine study IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, GM-CSF, IFN-y, and TNFalpha*	10 cc of peripheral blood in a SST tube	D-4, D0, D+2, D+4 and D+7 and then D+14 and then every planned follow-up appointment	Email: Robert Reger, robert.reger@nih.gov 24 hours prior to anticipated collection Please contact Robert Reger by e-mail (robert.reger@nih.gov) or at 301.594.8004, or Elena Cherkasova by email (cherkasovae@nhlbi.nih.gov) or at 301-827-3958 for questions about sample collections instructions.	-
Research Apheresis	4-5 L	-	Please contact Robert Reger by e-mail (robert.reger@nih.gov) or at 301.594.8004.	

***Research Blood can be collected +/- 1 day** except for D-4 and D0 which samples should be collected prior chemotherapy and HERV-E TCR transduced T-cell infusion**. Additional research blood may be requested at the discretion of the investigators as long as the amount of blood drawn does not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period. Obtaining blood samples may be discontinued for patients who had a disease progression at any time as per PI's discretion.**

9. BIOSTATISTICAL CONSIDERATIONS

Primary Objective: The primary objective of this study is to evaluate the safety of infusing escalating doses of HERV-E TCR transduced $CD8^+/CD34^+$ enriched T cells for the treatment of metastatic ccRCC.

Primary End-point: The primary endpoint will be the toxicity profile at each dose level captured using the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 except for hematological toxicities (Section 3.2).

Secondary End-points:
- To assess for tumor response using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 criteria
- To estimate the PFS and OS in such population.

Exploratory Studies:

- To determine the persistence, phenotype (including expression of checkpoint inhibitors) and function of HERV-E TCR transduced $CD8^+/CD34^+$ enriched T-cells circulating in patients post-infusion and to correlate findings with safety and efficacy (tumor response) of this therapy at each dose level.
- To study peripheral and tumor immune subsets and correlate findings with safety and efficacy (tumor response) of this therapy at each dose level.
- To evaluate changes in serum-soluble factors associated to T cell activation and migration and correlate findings with safety and efficacy (tumor response) of this therapy at each dose level.
- To determine the *ex-vivo* antigen-specific functional activity of HERV-E TCR transduced $CD8^+/CD34^+$ T-cells and correlate findings with safety and efficacy (tumor response) of this therapy at each dose level.
- To investigate the presence of HERV-E TCR transduced $CD8^+/CD34^+$ enriched T-cell in tumor tissue and its correlation with response to therapy.

Sample size

A standard '3+3' design will be used for this trial where 3 patients are treated at the first dose level. At a given dose level, if there are no DLT, the dose will be escalated to the next level. If there is one DLT in the first 3 patients treated at a dose level, an additional 3 patients will be treated at that level. If no additional DLTs are observed (for a total of 1 DLT in 6 patients), then the dose will be escalated.

If a level with 2 or more DLTs in 3-6 patients has been identified, 3 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. The MTD is defined as the highest dose at which 0 or 1 patient in six has experienced a DLT. We will report adverse events using the Common Terminology Criteria for Adverse Events version 4.0. If no DLTs are observed, we expect to treat up to 15 patients. If DLTs are observed, up to 6 patients may need to be treated at each cohort to establish the MTD. Thus, up to 24 patients may need to be enrolled onto the trial.

Statistical Methods

The planned analyses will include descriptive statistics on the incidence and severity of adverse events, such as the percentage of subjects with hematological or immunological toxicities. Since the risk of toxicity is assumed to increase with dosage, the isotonic regression method will be used to estimate probabilities of toxicity for different dose levels. A 21-days safety assessment period following cell administration will be conducted between each subject before the next subject in the cohort or the first subject in the next cohort would be treated.

The proportions of local adverse events, systemic adverse events and TRSAEs will be estimated using the sample proportions, with 95% confidence intervals. The time to adverse events will be analyzed using appropriate survival analysis methods such as Kaplan-Meier estimates.

The secondary endpoints will be plotted over time for each subject. The mean change of each variable over time will be estimated and compared among different dose cohorts. Ideally there will be a decrease in tumor mass on average when the cohorts are compared with respect to each of their doses of HERV-E TCR transduced $CD8^+/CD34^+$ enriched T-cells. Exploratory correlative analysis will be performed among biomarkers in peripheral blood and tumor tissue and tumor response.

If a subject fails to complete a cycle of protocol therapy for reasons other than toxicity, he/she will be deemed non-evaluable for determining the maximum tolerated dose and will be replaced. However, these subjects will be included in the evaluation of safety/toxicity. Toxicities will be tabulated by dose and grade.

Response will be first assessed at the day +30 time point following the HERV-E TCR transduced $CD8^+/CD34^+$ enriched T-cells infusion.

Protocol Stopping Rules

The study will be halted pending discussions with the FDA and NIH IRB regarding safety and the need for protocol revisions if any of the following conditions are met:

- If two DLTs, as described in Section DLT, occur in the first cohort of this study.
- If 2 or more patients develop a grade 3 or greater toxicity related to the cell product, except for:
  - Grade 3 metabolic laboratory abnormalities without significant clinical sequela that resolve to grade 2 or less within 7 days
  - Grade 3 fever
- 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 grade 3 autoimmunity, that cannot be resolved to less than or equal to a grade 2 autoimmune toxicity within 10 days, or any grade 4 or greater autoimmune toxicity.
- If one or more treatment related deaths occur due to the cell infusion, we will immediately stop study enrollment and promptly initiate discussions with the NIH IRB and FDA

Criteria for removal from protocol therapy and off study criteria

Off Treatment Criteria (Note: patients taken off-treatment will remain on study for the purpose of long-term follow-up data collection, RCR testing (recommended by FDA guidelines for patients treated with Retroviral Vector-Based Human Gene Therapy Products) and research sample at the discretion of the PI).

- Rapid disease progression prior to cell infusion that it would preclude the patient's ability to tolerate protocol therapy,
- Disease progression after HERV-E TCR transduced T cells infusion treatment
- Intercurrent illness that prevents administration of HERV-E TCR transduced CD8+/CD34+ enriched T-cells infusion,
- Unacceptable adverse event(s) that prevents administration of HERV-E TCR transduced CD8+/CD34+ enriched T-cells infusion,
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator, or
- Positive pregnancy test

Patients who are off treatment are to be followed until they meet the criteria for Off Study (see below). When off treatment, long-term follow-up data on clinical data related to time-endpoints or FDA-mandated evaluation for patients treated with Retroviral Vector-Based Human Gene Therapy Products will be collected during this period will be collected unless consent is withdrawn.
RCR testing will be performed as per FDA guidelines for patients treated with Retroviral Vector-Based Human Gene therapy Products and research sample will be collected at the discretion of the PI.

Off Study Criteria

- The patient voluntarily withdraws,
- There is significant noncompliance,

- General or specific changes in the patient's condition render continued participation the patient unacceptable for further follow up in the judgment of the investigator, or
- Death
- Completion of protocol

10. DATA and SAFETY MONITORING

Safety Monitoring Principal Investigator: Accrual, efficacy and safety data will be monitored by the PI

NIH IRB: Prior to implementation of this study, the protocol and the proposed patient consent forms will be reviewed and approved by the properly constituted IRB operating according Title 21 Code of Federal Regulations (CFR) Part 56. This committee will also approve all amendments to the protocol or informed consent, and conduct continuing annual review so long as the protocol is open to accrual or follow up of subjects.

DSMB: The NHLBI Data Safety and Monitoring Board (DSMB) will review the protocol at least annually. A progress report will be forwarded to the DSMB at these times and their recommendations will be expeditiously implemented. The DSMB may recommend early termination of the study for considerations of safety and efficacy.

FDA: (IND 17690) An annual progress report, any amendments to the protocol, and any change in the status of the protocol will be forwarded to FDA.

10.1 Definitions

The PI or designee will refer to HRPP Policy 801 "Reporting Research Events" for definitions.

10.2 Adverse Event Management

All abnormal physical exam findings, laboratory events and other events defined in CTCAE v5.0 will be collected. The AEs will be attributed (unrelated, unlikely, possibly, probably or definitely) to study medication and/or disease. This study will utilize the CTCAE version 5.0 for toxicity and adverse event reporting. A copy of the CTCAE version 5.0 can be downloaded from the https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

Baseline symptoms will be collected starting on the day the subject signs the screening protocol informed consent. However, we will use baseline symptoms and laboratory results collected during the clinical visit and laboratory tests prior to starting chemotherapy for the purpose of reporting treatment-related adverse event.

Adverse events of any grade will be collected during the first year after cell infusion or until a subject is determined to have disease progression, at which time, the subject will remain on study only for gene therapy related long-term follow up. After one year, or after a subject is determined to have disease progression, no adverse events will be collected, unless they are thought to be at least possibly related to cell infusion.

10.2.1 Grading of Adverse Events

1	**Mild**	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	**Moderate**	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*
3	**Severe**	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
4	**Life-threatening**	Life-threatening consequences; urgent intervention indicated.
5	**Death**	Death related to AE

Hospitalization (overnight admission) for routine supportive care (platelet or red blood cell transfusions) or admission from the NIH inpatient unit to the NIH ICU for routine monitoring will not be reported as a serious adverse event.

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

10.3 NIH-Intramural IRB and CD reporting

Expedited Reporting

Events requiring expedited reporting will be submitted to the IRB per Policy 801 "Reporting Research Events".

Reports to the IRB at the time of Continuing Review:

The PI or designee will refer to HRPP Policy 801 "Reporting Research Events" to determine IRB reporting requirements.

Reports to the CD:

The PI or designee will refer to NHLBI DIR Policy to determine CD reporting requirements and timelines.

10.5 Reporting of Pregnancy

Subjects who become pregnant during the first year on study should discontinue any study drugs that may adversely affect the pregnancy. The investigator, or his/her designee, will collect pregnancy information on any subject who becomes pregnant while participating in this study.

The sponsor-investigator, or his/her designee, will submit pregnancy information to the NHLBI Clinical Director and NIH IRB within two weeks of learning of a subject's pregnancy. Information on the status of the mother and child will also be reported to the CD and NIH IRB no longer than 6 to 8 weeks following the estimated delivery date. Any premature termination of the pregnancy will be reported. While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded and reported as an AE or SAE.

10.6 IND Sponsor Reporting Criteria

Events will be submitted to Dr. Richard Childs, IND Sponsor Authorized Representative at:
Richard Childs, M.D.,
Bldg 10, CRC, Room CRC 3-5332
Phone: (301) 594-8008

10.7 FDA Reporting Criteria

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

The Sponsor will notify the FDA 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:

- 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.

Copies of all IND Safety Reports submitted to the FDA per 21 CFR 312.32 will also be submitted to CTEP: CTEPSupportAE@tech-res.com.

10.8 FDA Annual Reports (Refer to 21 CFR 312.33)

The study Sponsor 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 as indicated in 21CFR 312.33, and any associated FDA correspondences regarding the IND annual report.

10.9 Reports to the DSMB

Reports of serious adverse events that are **unexpected and suspected** will also be forwarded as soon as possible and no later than seven (7) days in the case of death or life-threatening serious adverse events or within fifteen (15) days after the occurrence of all other forms of serious adverse events to the Data and Safety Monitoring Board (DSMB). A summary of all SAEs will be included for review semiannually or annually by the DSMB.
If the serious adverse event is thought to be due to the experimental component of the protocol, accession to the protocol will be stopped until a full discussion with the IRB has been held.

10.10 Routine Adverse Event Recording

All adverse events will be recorded in the patient's medical record. Following enrollment, adverse events will be reviewed by the research nurse and principal investigator and captured into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. All events occurring during the treatment phase of the study will be followed until resolution or stabilization.

10.11 Clinical trial monitoring

As per ICH-GCP 5.18 and FDA 21 CFR 312.50 clinical protocols are required to be adequately monitored by the study sponsor.
The monitoring of this study will be conducted by Clinical Research Associates (CRAs)/monitors employed by an independent contract organization working under an agreement with NHLBI to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol.

The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent form (ICF) and documentation of the ICF process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare abstracted information with individual subjects' records and source documents (subject's charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections-OHRP), FDA and applicable guidelines (ICH-GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit. The investigator (and/or designee) will make study documents (e.g., consent forms and pertinent hospital or clinical records readily available for inspection by the local IRB, the FDA, the site monitors, and the NHLBI staff for confirmation of the study data.

11. DATA COLLECTION AND EVALUATION

During the course of study participation, specimens will be collected for correlative studies.

Research Sample Collection: The quantities of blood to be drawn for research purposes will be consistent with the Clinical Center policy as provided in Medical Administrative Series (MAS) 95-9. The amount of blood that may be drawn from adult patients and volunteers for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight week period. For pediatric patients, no more than 5 mL/kg may be drawn for research purposes in a single day, and no more than 9.5 mL/kg may be drawn over any eight-week period.

12. BIOSPECIMEN AND DATA MANAGEMENT PLAN

Biospecimen Tracking and Management

Samples will be ordered and tracked through the CRIS research screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record. Specimens and their derivatives (e.g., genomic material, cell lines) will be coded and stored in conformity with DIR Policy (e.g., BSI). Coded biospecimens may be sent to collaborators outside of the NIH with IRB approval in accordance with applicable NIH and DIR Policy for sharing research resources, including an executed material transfer agreement (MTA). Biospecimens with subject personal identifiers

may be sent to associate investigators and collaborators outside of the NIH only after approvals of both NIH and local IRBs, an executed reliance agreement with NIH IRB, or an extension of the NIH's FWA through an Individual Investigator Agreement.

Loss or destruction of samples: Should we become aware that a major breech in our plan for tracking and storage of samples has occurred, the IRB will be notified.

Data management

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. Laboratory values from referring home physicians will be entered into the system. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts to ensure that data is verifiable and evaluable.

Research data will be prospectively collected by authorized Investigator personnel and entered into an NHLBI-approved database. The database will consist of the study specific set of electronic CRFs (e-CRFs) used for capturing, managing and reporting clinical research data.

The database will maintain complete data records on each research subject. Subjective and objective patient experiences during the duration of the study will be documented in the patient medical record notes. These protocol notes will serve as the primary source material from which data will be collected and research analyses will be performed. Any pertinent supplementary information obtained from outside laboratories, outside hospitals, radiology reports, laboratory reports, or other patient records will be used as additional source for data collection.

Identifiable data will not be sent outside NIH without prior IRB approval or appropriate conditions for disclosure outlined in the executed CDA or MTA.

CDUS-Abbreviated reporting/monitoring to CTEP

This study will be monitored by the Clinical Data Update System (CDUS) Version 3.0. Cumulative protocol- and
patient-specific CDUS data will be submitted electronically to CTEP on a quarterly basis by FTP burst of data. Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting data using the CDUS can be found on the CTEP Web site (http://ctep.cancer.gov/reporting/cdus.html).

CDUS-Abbreviated reporting: no adverse event reporting (routine or expedited) is required to be reported via CDUS, but expedited adverse events are still required to be submitted via CTEP-AERS.

End of study procedures: Data will be stored in locked cabinets and in a password protected database until it is no longer of scientific value.

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

Publication policy: Given the research mandate of the NIH, subject data including the results of testing and responses to treatment will be entered into an NIH-authorized and controlled research database. Any

future research use will occur only after appropriate human subject protection institutional approval such as prospective NIH IRB review and approval or an exemption from the NIH Office of Human Subjects Research Protections (OHSRP).

Data sharing and future use of data

Research data may be shared with qualified non-collaborator recipients following publication of the primary research results after removal of PII and IRB approval. Future research use of data not defined in the research protocol may occur only after IRB review and approval or an exemption from the NIH OHSRP. Refusal of a research subject participant to permit future use of data--other than required in the protocol or by the FDA--will be honored. Limitations in data sharing and future use of data due to contractual obligations (e.g., CRADAs) or intellectual property proceedings (such as patent filings) will be honored.

Future use of biospecimens

Following analyses of biospecimens for primary research purposes, remaining samples suitable for future research will be stored in the Principal Investigator's laboratory freezers in a coded manner to ensure data protection.

Any future research use of biospecimens not defined in the protocol in which NHLBI investigators are engaged in research (e.g., they are undertaking research activities and hold the key that identifies research subjects) requires IRB review and approval. Coded biospecimens (NHLBI investigators hold the key that identifies research subjects) to be shared outside of NIH for future research use where results will not be returned to the Principal Investigator does not require IRB review or approval.

13. RESPONSE CRITERIA

Response will be documented by physical exam and/or noninvasive imaging using techniques that demonstrate lesions. Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1).[143] Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria. For the purposes of this study, patients should be re-evaluated for response every 6-8 weeks. In addition to a baseline scan, confirmatory scans should also be obtained at least 4 weeks following initial documentation of objective response.

If a patient is classified as having PD at the first post-baseline tumor assessment (D+30), then confirmation of PD by a second scan in the absence of rapid clinical deterioration is required on D+60. The definition of confirmation of progression at the first post-baseline tumor assessment represents an increase in tumor burden ≥20% compared to the baseline tumor size confirmed at two consecutive time points at least 4 weeks apart. It is recommended that this be done at the discretion of the investigator because follow-up with observation alone may not be appropriate for patients with a rapid decline in performance status. However, we anticipate that confirmation of PD will allow for the capture of all observed responses, as late-responses were observed in patients with RCC months after transplantation (median of 129 days), and often followed the withdrawal of cyclosporine and the establishment of complete donor–T-cell chimerism.[33] Otherwise, the definition of disease progression represents an increase in tumor burden of ≥20% compared with the nadir. Confirmation of progression as describe above will be performed at the PI's discretion after D+90 time point.

If a patient is classified as having CR, PR or SD at the first post-baseline tumor assessment (D+30), then; next tumor assessment will take place on D+90 unless otherwise clinically indicated.

Evaluation of Target Solid Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum of diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum of diameters while on study.

Evaluation of Bony Lesions in Patients with Isolated Bone Metastases

Complete Response (CR): Normalization of all bony lesions (target and nontarget) to SUL less than mean liver SUL and equal to normal surrounding tissue SUL. Verification with follow-up study in 1 month if anatomic criteria indicate disease progression.

Partial Response (PR): > 30% decrease in SUL peak; minimum 0.8 unit decrease. Verification with follow-up study if anatomic criteria indicate disease progression.

Progressive Disease (PD): > 30% increase in SUL peak (minimum 0.8 unit increase in SUL peak), > 75% increase in TLG of the 5 most active lesions, Visible increase in extent of FDG uptake, or new lesions. Verification with follow-up study if anatomic criteria indicate complete or partial response.

Stable Disease (SD): Does not meet other criteria.

Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis). Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions.
Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase. Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

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.

For Patients with Measurable Tumors (i.e., Target Disease)

Target Lesions	Non-Target Lesion	New lesions	Overall Response	Best Overall Response when Confirmations is Required*
CR	CR	No	CR	≥ 4 weeks. Confirmation*
CR	Non-CR/Non-PD	No	PR	≥ 4 weeks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥ 4 weeks from baseline**
PD	Any	Yes or No	PD	No prior SD,PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesions
**Only for non-randomized trials with response as primary response
***In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.
Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as " symptomatic deterioration". Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (i.e. Non-Target Disease)

Non-Target Lesions	New lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	No-CR/non-PD*
Not all evaluated	No	Not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

*'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as a endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or 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 CR is measured from the time measurement criteria are first met for CR until the first date that progressive 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, including the baseline measurements.

14. HUMAN SUBJECT PROTECTIONS

Rationale for Subject Selection

All patients between the ages of 18 and 75 years with eligible diseases will be considered for the protocol. No subjects will be excluded from participation based on gender, race or ethnicity. Due to the experimental nature of the study and the potential serious risk and limited information of its effects in elderly patients, subjects greater than 75 years will not be eligible.

We anticipate distribution by race, gender, and age will be similar to our concurrent solid tumor transplant protocol (99-H-0064) as follows:

- By gender: females (48%), and males (52%).
- By age: 24 to 67 years of age (median 47),
- By race: Asian (2%), Black (7%), Hispanic (3%), and white (88%).

HIV-positive patients are excluded because the experimental treatment being evaluated in this protocol depends on an intact immune system. Patient who are HIV seropositive can have decreased immune competence and thus be less responsive to the experimental treatment and more susceptible to its toxicities. Palliative radiation therapy to areas of metastatic tumors is commonly used to treat patients with metastatic cancer. Such patients often maintain a good performance status and sufficient hematopoietic reserve after such radiation making them candidates for subsequent investigational trials or other standard treatment modalities.

Pregnant or nursing mothers are excluded because effects of chemotherapy, IL-2 and HERV-E TCR transduced $CD8^+/CD34^+$ enriched T-cells on the developing human fetus are unknown. For this reason, women of child-bearing potential should agree to use adequate contraception prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

Exclusion of Pregnant Women

Pregnant women are excluded from accrual to eliminate the possibility of drug effects on a developing fetus. Subjects are required to use effective contraceptive measures to prevent pregnancy within one year of participation in the study.

Participation of Children

Patients less than 18 years of age will not be eligible in this study due to the rarity of the eligible diseases in the pediatric population and because of the potential serious risks and limited information of the protocol regimen's effects in children. Should the results of this trial demonstrate efficacy, consideration will be given to expanding accrual to this patient population.

Participation of subjects unable to give consent

Informed consent for adult research participants unable to provide consent
If there is an unexpected enrolment of a research participant unable to provide informed consent, we will follow the procedure for obtaining informed consent from a legally authorized representative (LAR) per NIH HRPP Policy 403 (Appendix D).

Justification for inclusion:

This research provides the prospect of direct benefit; therefore, inclusion is justified. The benefits to the participants could be regression of metastatic kidney cancer resulting in improved quality of life and also decreased morbidity and mortality from oncological complications (i.e. cord compression, dyspnea, pain). Potentially, oncological complications and treatment with other toxic therapies could also be avoided or postponed. Not allowing participants who cannot provide consent would deny them the potential benefits this protocol offers for their disease. There are no plans to include institutionalized participants.

Consent and Assent:

Procedures to determine capacity: If documentation of decision making capacity is not present in the medical record or the investigator questions the decision making capacity of the individual, then the Ability to Consent Assessment Team (ACAT) (301-496-9675 or 301-496-2429) will be contacted to make the determination.

Procedures to obtain assent and documentation of assent or dissent:

The informed consent discussion will include the individual unable to provide informed consent along with LAR. The individual unable to provide informed consent will be asked if they agree to participate in the research and this will be documented in the medical record. Assent from decisionally impaired subjects will be obtained orally and documented in the patient's file. Subject dissent will be honored.

For Patients that become cognitively impaired during treatment

Re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section 4.2), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. **Note:** The PI or AI will contact the NIH ACAT for evaluation. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MEC Policy 87-4 for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

No Competition among Branch Protocols

Strategies for patient recruitment at the NIH: The study may use the following strategies of recruitment including:

- ClinicalTrials.gov website
- Clinical Center Research Studies ("Search the Studies") website
- Engagement with patient advocacy groups such as Kidney Cancer Association, American Cancer Society, Action to Cure Kidney Cancer, Inspire Kidney Cancer Association Support Group, and the American Cancer Fund
- National Heart, Lung and Blood Institute (NHLBI) patient recruitment website
- Twitter messages and chats with study investigators
- Facebook Posts through official NIH accounts
- Google Ad Words

- Use of Clinical Center Office of Patient Recruitment services including creation and distribution of study flyers and information through pre-existing recruitment avenues such as the NIH recruitment listserv.

The DIR Patient Recruitment Office (PRO) will work with study investigators to ensure accrual goals are being met.

- Biannual reviews will be performed by investigators and the PRO.
- If recruitment goals are lagging, an enhanced plan of recruitment will be triggered, which may include a communication blitz to known and newly contacted hematologists and oncologists throughout the country; additional Twitter feeds and chats, Facebook posts and recirculation of updated and/or new patient recruitment flyers. All recruitment materials and tools will use IRB-approved language and information to include standard recruitment contacts.

Oncologists and Renal Cancer specialists throughout the country will be actively informed about the protocol by letter.

No subjects will be recruited at Loyola University, Chicago.

Reimbursement for protocol participation, travel, food, and lodging: There is no payment or compensation to subjects for their participation in this protocol. Reimbursement for travel, food, and lodging will be consistent with NHLBI DIR Travel and Lodging Compensation of Clinical Research Subjects policy or institutional guidelines.

15. RISK and DISCOMFORTS

15.1 Related to cyclophosphamide

Cyclophosphamide toxicities includes:
1) Nausea and vomiting-variable; symptomatically improved with standard anti-emetics and/or benzodiazepines [e.g., lorazepam].
2) Water retention–cyclophosphamide may rarely provoke the syndrome of inappropriate antidiuretic hormone secretion and resultant hyponatremia, usually manifested 12-48 hours after IV administration.
3) Cardiomyopathy-cyclophosphamide may cause severe, sometimes lethal, hemorrhagic myocardial necrosis or congestive cardiomyopathy. Patients may present with congestive cardiomyopathy as late as 2 weeks after the last dose of cyclophosphamide. In an attempt to minimize this complication, patients with significant cardiac dysfunction are excluded from this protocol [see patient eligibility]. Congestive failure is managed according to standard medical therapeutics. Cardiac toxicity with the dose of cyclophosphamide administered on this protocol is extremely unlikely.
4) Hemorrhagic cystitis–this is a serious, potentially life-threatening complication related to injury of the bladder epithelium by cyclophosphamide metabolites.
5) Less common but serious complications include pulmonary fibrosis and secondary malignancies. Less common but reversible toxicities include alopecia and skin rash.

15.2 Related to fludarabine

Fludarabine toxicities include myelosuppression (dose limiting toxicity), fever, nausea, vomiting, stomatitis, diarrhea, gastrointestinal bleeding, anorexia, edema, skin rashes, myalgia, headache, agitation, hearing loss, transient episodes of somnolence and fatigue, anemia, auto-immune thrombocytopenia, paresthesias, peripheral neuropathy, renal, and pulmonary toxicity (interstitial pneumonitis). Severe fatal CNS toxicity presenting with loss of vision and progressive deterioration of mental status were encountered

almost exclusively after very high doses of fludarabine monophosphate. Such toxicity has only rarely been demonstrated at the 25-30 mg/m2/day dosage of fludarabine. Very rarely described complications include transfusion-associated graft-versus-host disease, thrombotic thrombocytopenic purpura, and liver failure.

Tumor lysis syndrome following fludarabine administration has been observed, especially in patients with advanced bulky disease. Opportunistic infections (protozoan, viral, fungal, and bacterial) have been observed post-fludarabine, especially in heavily pre-treated individuals, and in individuals receiving fludarabine combined with another agent.

15.3 Related to Interleukin-2 (IL-2, aldesleukin)

Interleukin-2 boxed warnings. The boxed warnings are for high dose interleukin-2 (600,000 IU/kg every 8 hours). For the average adult (70 kg) the total daily dose would be 126 $x10^6$IU/day. This protocol calls for moderate dose interleukin-2 (2,000,000 IU/m^2 q 12 hours on day 0 through day +6). For the average adult (1.73 m^2) the total daily dose would be 7 $x10^6$ IU/day or roughly 6% of the high dose amount, therefore, we do not expect any of the boxed warnings as detailed below:

15.3.1 Boxed Warnings related to interleukin-2:

Cardiac and pulmonary: Therapy with interleukin-2 should be restricted to patients with normal cardiac and pulmonary functions as defined by thallium stress testing and formal pulmonary function testing. Extreme caution should be used in patients with a normal thallium stress test and a normal pulmonary function test who have a history of cardiac or pulmonary disease.

Hospital setting: Interleukin-2 should be administered in a hospital setting under the supervision of a qualified physician experienced in the use of anti-cancer agents. An intensive care facility and specialists skilled in cardiopulmonary or intensive care medicine must be available.

Capillary Leak Syndrome: Interleukin-2 administration has been associated with capillary leak syndrome (CLS) which is characterized by a loss of vascular tone and extravasation of plasma proteins and fluid into the extravascular space. CLS results in hypotension and reduced organ perfusion which may be severe and can result in death. CLS may be associated with cardiac arrhythmias (supraventricular and ventricular), angina, myocardial infarction, respiratory insufficiency requiring intubation, gastrointestinal bleeding or infarction, renal insufficiency, edema, and mental status changes.

Neutropenia: Interleukin-2 treatment is associated with impaired neutrophil function (reduced chemotaxis) and with an increased risk of disseminated infection, including sepsis and bacterial endocarditis. Consequently, preexisting bacterial infections should be adequately treated prior to initiation of interleukin-2 therapy. Patients with indwelling central lines are particularly at risk for infection with gram-positive microorganisms. Antibiotic prophylaxis with oxacillin, nafcillin, ciprofloxacin, or vancomycin has been associated with a reduced incidence of staphylococcus infections.

CNS Toxicity (Lethargy, somnolence, coma): Interleukin-2 administration should be withheld in patients developing moderate to severe lethargy or somnolence; continued administration may result in coma.

15.3.2 Adverse Events related to interleukin-2:

Adverse events associated with interleukin-2, while potentially serious, are generally predictable, manageable, reversible and more commonly occur with high dose therapy. Patients treated with interleukin-2 may experience significant side effects; but interleukin-2 is acute therapy, with most patients receiving

only 1 or 2 courses of treatment. Nearly all toxicities are self-limiting, rapidly reversible or improve within 2 to 3 days of discontinuing therapy. Long-term sequelae associated with interleukin-2 are extremely rare.

The following common adverse events are based on 525 patients (255 with metastatic renal cell carcinoma and 270 with metastatic melanoma) treated with the recommended interleukin-2 infusion dosing regimen:

Event	**Grade 1-4 Seen in ≥ 30% of Patients (n=525)**	**Grade 4 Seen in > 1% of Patients (n=525)**
Hypotension	71%	3%
Diarrhea	67%	2%
Oliguria	63%	6%
Chills	52%	-
Vomiting	50%	1%
Dyspnea	43%	1%
Rash	42%	-
Bilirubinemia	40%	2%
Thrombocytopenia	37%	1%
Nausea	35%	-
Confusion	34%	1%
Creatinine increase	33%	1%
Anuria	-	5%
Respiratory	-	3%
Coma	-	2%
Infection	-	1%
Sepsis	-	1%
Cardiovascular disorder	-	1%
Myocardial Infarct	-	1%
Psychosis	-	1%

Other adverse events that could occur because of IL-2 are muscle and joint pain.

15.4 Related to the apheresis procedure

The apheresis procedures will be performed in accordance with standard apheresis donation policies and procedures operative in the Department of Transfusion Medicine and will be following the Blood Donor Standards of the American Association of Blood Banks and the rules and regulations of the Food and Drug Administration. Adverse reactions to apheresis procedures are rare, but include:

- Pain and hematoma at the needle placement site.
- Vasovagal episodes, characterized by transient hypotension, dizziness, nausea and rarely, syncope are seen in less than 2% of the procedures. Hypotension secondary to volume depletion is known for the rare potential for a cerebrovascular or cardiovascular event.

- Cutaneous or circumoral parasthesias, chills, nausea, heartburn and rarely muscle spasms may result from the use of citrate anticoagulant used to prevent clotting in the extracorporeal circuit. Citrate reactions are usually relieved by slowing the rate of the anticoagulant infusion and by administering oral calcium carbonate tablets or with intravenous calcium gluconate.

Prior to each apheresis, the potential risks associated with the procedure will be explained to the subject and a separate informed consent obtained.

15.5 Related to blood draws

No major risks are involved with blood draws. Minor complications including bleeding, pain, and hematoma formation at the site of the blood draw. Infection may rarely occur.

15.6 Related to central line placement

It is estimated that about 50% of subjects will require intravenous line placement, typically in the femoral vein to successfully complete apheresis. All subjects who do not have a chronic indwelling central venous line will have a single-lumen PICC intravenous line (or other central line if arm veins are sub-optimal) placed by the Venous Access Device (VAD) service of the Clinical Center. This line will aid with blood draws, medication infusion and ensure IV infusion of the expanded HERV-E TCR-expressing $CD8^+/CD34^+$ enriched T cells. If a PICC line cannot be placed, subjects may have a midline catheter placed as an alternative to a central venous catheter.

Intravenous line placement carries a small risk of bleeding, bruising or pain and a very low risk of accidental injury to the adjacent artery and nerve. Some subjects may experience a vasovagal reaction (lightheadedness, or rarely, fainting due to temporary lowering of blood pressure). Using only trained experienced MICU/VAD staff for the procedure minimizes these risks.

15.7 Related to metastatic site biopsy (when indicated)

A fine-needle or core needle aspiration biopsy puts a thin needle into the lymph node or other visceral metastatic sites and removes cells to look at. Subjects will feel only a quick sting from the local anesthesia used to numb the skin and may feel some pressure from the biopsy needle. After a fine-needle aspiration biopsy or core needle biopsy, the site may be tender for 2 to 3 days. An open biopsy of a lymph node makes a cut in the skin and removes the lymph node.

15.8 Related to Thoracentesis

Potential complications from an ultrasound-guided thoracentesis, include pain at the puncture site (2.7%), bleeding (hematoma, hemothorax, or hemoperitoneum) (0.4%), pneumothorax (2.5%), shortness of breath (1.0%) empyema, soft-tissue infection, spleen or liver puncture, re-expansion pulmonary edema when a large amount of fluid is removed (0.2%), vasovagal events (0.6%), and adverse reactions to local anesthetic or topical antiseptic solutions. The most common clinically important complication is pneumothorax, with an incidence of 2.5%. When a pneumothorax occurs, it is usually small and often requires no additional therapy. Tube thoracostomy is required in only 0.8% of patients. Liver or spleen puncture may occur when the patient is not sitting absolutely upright, because movement towards recumbency causes cephalad migration of the abdominal viscera. Nonetheless, even if the liver or spleen is punctured by a small-bore needle, the outcome is generally favorable if the patient is not receiving anticoagulants and does not have a bleeding diathesis. There is also a risk for infection, anaphylaxis, and sustained hypotension (mean arterial pressure < 60 mm Hg unresponsive to intravenous fluids).

15.9 Related to Paracentesis

The risks of an abdominal paracentesis include pain at the puncture site, bleeding (hematoma or hemoperitoneum), pneumothorax bacterial peritonitis, soft-tissue infection, sepsis, spleen or liver puncture, vasovagal events and adverse reactions to local anesthetic or topical antiseptic solutions, anaphylaxis, and sustained hypotension (mean arterial pressure < 60 mm Hg unresponsive to intravenous fluids).

15.10 Related to imaging studies (when indicated)

15.10.1 Chest X-ray: The risks associated with x-rays are very small. For example, one chest x-ray has the same increased risk of death (due to cancer) as smoking 1.4 cigarettes or drinking ½ liter of wine. On average, all of us receive an equivalent dose of x-rays every 30 days from nature. However, the more radiation you receive over the course of your life, the greater the risk of developing cancerous tumors or of inducing changes in genes. The changes in genes could cause abnormalities or disease in your future offspring. We do not expect the radiation in this study to greatly increase these risks, but we do not know the exact increase in such risks. Additionally, we do not stipulate x-rays to be done as routine monitoring and they would only be done at the PI discretion as medically indicated.

15.10.2 CT scan: CT (computed tomography), sometimes called CAT scan, uses special x-ray equipment to obtain image data from different angles around the body and then uses computer processing of the information to show a cross-section of body tissues and organs. Oral and/or intravenous contrast agents will be used and are usually well tolerated. However, some subjects will experience allergic reactions to intravenous contrast. To lower the risk of allergic reactions, low allergenic contrast agents are administered at NIH Clinical Center. In addition, subjects will be advised that approximately 2-7% of patients who receive contrast agents will experience a temporary reduction in kidney function lasting up to 2 weeks following infusion and that in rare instances, permanent renal damage can result from the use of the IV contrasting agent. Therefore, in subjects with impaired kidney function, we will not use intravenous contrast.

15.10.3 F18-FDG-PET CT: Positron emission tomography or "PET scan" is an alternative approach to monitoring disease activity. Rapidly growing tissue, such as cancer, take up and use more sugar than does normal tissue. To detect areas of rapidly growing tissue we will inject a radioactive sugar (FDG) into the body through a vein. Then by means of a special scanner which senses the uptake of the radioactive sugar, images will be produced identifying areas of rapidly growing tissue in the body.

The subject will be asked to come to the NIH 2 hours before the PET scan. We will place one small plastic tube (called a catheter) into a vein into the arm. We will use this tube for injections of the radioactive sugar. Subjects will be asked to wait for 60 min in the Nuclear Medicine Department. After 60 minutes, we will ask the subject to lie down on the scanner bed. The scanning procedure will take in total 2 – 3 hours to complete. He/she will be asked to lie still during the scan. We will monitor the subject throughout the procedure. When the scanning is completed, the subject will be asked to go to the bathroom to void which will minimize radiation exposure. All women of child bearing potential will be required to sign and have a pregnancy test no more than 24 hours before the PET scan tracer injection.

Subjects may feel a quick sting or pinch when the IV is inserted. Most of the tracer will be flushed from the body within 6 to 24 hours. Allergic reactions to the tracer are very rare. In rare cases, some soreness or swelling may develop at the IV site where the radioactive tracer was put in. Subjects will be advised to apply a moist, warm compress to his/her arm.

15.10.4 Magnetic resonance imaging (MRI): Magnetic resonance imaging (MRI) uses a strong magnetic field and radio waves to obtain images of body organs and tissues. The MRI scanner is a metal cylinder surrounded by a strong magnetic field. During the MRI, the subject lies on a table that can slide in and out of the cylinder. Scanning time varies, with most scans lasting between 45 and 90 minutes. While in the scanner subjects will hear loud knocking noises, and may request earplugs to muffle the sound. Subjects with a fear of confined spaces may become anxious during this procedure. MRI staff and patients are able to communicate at all times during the scan, and may ask to be moved out of the machine at anytime they become uncomfortable. If contrast is ordered, Gadolinium, an FDA approved medication will be used to improve MRI images. About 98% of patients receiving gadolinium have no symptoms related to the injection of this medication. Mild symptoms that may occur include: coldness in the arm at injection, a metallic taste, headache, and nausea. In an extremely small number of patients, more severe symptoms have been reported including: shortness of breath, wheezing, and lowering of blood pressure. The FDA has issued a warning that administration of gadolinium, the contrast imaging agent that may be used in this protocol, has been associated with development of a disease called nephrogenic systemic fibrosis (NSF). The syndrome is rare (approximately 200 cases reported worldwide as of December 2006 out or several million administrations of gadolinium), but disabling and in some cases, fatal. All cases to date have occurred in patients with severe renal disease, including patients on dialysis. We will ask the patient whether they have or have had kidney disease or diabetes, whether they take diuretics (water pills) for any medical condition and whether they have received x-ray dye or drugs recently that might affect their kidney function. Depending on their history and creatinine levels, it will be determined whether they may receive gadolinium.

On December 19, 2017, the FDA is requiring a new class warning and other safety measures for all gadolinium-based contrast agents (GBCAs) for magnetic resonance imaging (MRI) concerning gadolinium remaining in patients' bodies, including the brain, for months to years after receiving these drugs. Gadolinium retention has not been directly linked to adverse health effects in patients with normal kidney function, and FDA has concluded that the benefit of all approved GBCAs continues to outweigh any potential risks. However, after additional review and consultation with the Medical Imaging Drugs Advisory Committee, FDA is requiring several actions to alert health care professionals and patients about gadolinium retention after an MRI using a GBCA, and actions that can help minimize problems. These include requiring a new patient Medication Guide, providing educational information that every patient will be asked to read before receiving a GBCA. FDA is also requiring manufacturers of GBCAs to conduct human and animal studies to further assess the safety of these contrast agents.

In response to the December 2017 MedWatch warning on gadolinium accumulation from the FDA, the healthy subjects may undergo no more than two gadolinium exposures during a 12-month period, per NHLBI policy.

In accordance with the FDA Drug Safety Communication of 05/16/2018, the Medication Guide for gadobutrol (or other macrocyclic gadolinium contrast agent if applicable) will be made available to all subjects with scans that will involve gadolinium-based contrast agent administration.

15.10.5 Bone scan: A bone scan is a nuclear scanning test that identifies new areas of bone growth or breakdown. It can be done to evaluate damage to the bones, detect cancer that has spread (metastasized) to the bones, and monitor conditions that can affect the bones (including infection and trauma). A bone scan can often detect a problem days to months earlier than a regular X-ray test. For a bone scan, a radioactive tracer substance is injected into a vein in the arm. The tracer then travels through the bloodstream and into the bones. A special camera (gamma) takes pictures of the tracer in the bones. This helps show cell activity and function in the bones. Areas that absorb little or no amount of tracer appear as dark or "cold" spots,

which may indicate a lack of blood supply to the bone (bone infarction) or the presence of certain types of cancer. Areas of rapid bone growth or repair absorb increased amounts of the tracer and show up as bright or "hot" spots in the pictures. Hot spots may indicate the presence of a tumor, a fracture, or an infection.

15.10.6 Radiation Risks: The amount of radiation subjects may receive from the research scans in this study is 21 rem of radiation annually. All female subjects will receive pregnancy testing prior to radiation exposure.

15.11 Related to cardiac function

15.11.1 Electrocardiogram (EKG): EKG is a test that measures the electrical activity of the heartbeat. With each beat, an electrical impulse (or "wave") travels through the heart. This wave causes the muscle to squeeze and pump blood from the heart. A technician will put patches (electrodes) on the chest, arms and legs.

The electrodes are soft and don't cause any discomfort when they're put on or taken off by the technician. The machine only records the EKG. It doesn't send electricity into the body. There's no pain or risk associated with having an electrocardiogram.

15.11.2 Echocardiogram: This test involves holding a small probe against the subject's chest wall to obtain pictures of the heart. The probe uses sound waves to detect the structures of the heart and does not involve any radiation. It has no known side effects.

15.12 Safety Considerations for HERV-E TCR transduced CD8+/CD34+ enriched T cell

The risks of the infusion of large numbers of retrovirally modified tumor reactive T-cells fall into 3 general categories. The first category of toxicity is cytokine-release-type toxicities such as high fevers, tachycardia, transient abnormalities of liver function, and hypotension. These cytokine-release-type toxicities have been detected rarely in other clinical trials of TCR T cells during the first 2 weeks after modified tumor reactive T-cells are infused.

The second possible category of toxicity is direct damage to normal tissues by the retrovirally modified tumor reactive T-cells. This could happen because of unexpected cross-reactivity of the anti-HERV-E TCR with proteins other than HERV-E in vivo. This trial will be the first to target an antigen derived from a HERV-E, so cross-reactivity with normal proteins is not inconceivable. We have performed extensive testing of HERV-E expression by RT-PCR of cells derived from a variety of human tissues and other malignant cell lines and we have not seen expression of this HERV-E in these specimens. Rooney *et al*. performed extensive testing of ERV-4 (also called HERV-E) by RNA-Seq-derived with minimal to undetectable expression in normal tissues or other malignancies and elevated expression in clear cell RCC samples.[98] Amino acid scanning approach also was performed that demonstrated no known naturally occurring peptides with high sequence homology to CT-RCC-1 making alternative target peptides for the HERV-E A11 TCR unlikely (Table 9).

Other potential type of toxicities that can occur in a clinical trial of T-cell gene therapies include occurrence of accidental exposure to the agent, replication-competent retrovirus or transformation of T cells caused by insertional mutagenesis. The transgenes expressed by the HERV-E/CD34t SAMEN retroviral vector integrate stably into the genome of cells infected with the GALV pseudotyped retrovirus. Given the CD34 selection, the T cell cultures produced are highly enriched for transduced T cells. The transduction

conditions were developed to limit copy number to an average of <5 integrated copies per cell as mandated by the FDA (usually between 1 and 2 copies per cell). Despite the low copy number, there are potential risks of using the retroviral vector and the transduced T cells which are as follows: a) Accidental exposure to the agent by contact with the skin, inhalation, injection or through a puncture could result in inflammation or an immune reaction to the virus or other components in the virus preparation, b) insertion of the provirus into cells of the host at a point in genome where it can disrupt normal cell function leading to clonal expansion or malignant transformation, and/or c) recombination in the host cells between the virus and an defective endogenous retrovirus in the host cell leading to replication competent virus.

While these are formal possibilities, the risks are considered to be small. In the case of accidental exposure to the agent, appropriate blood borne pathogen precautions will be adopted by NIH staff. Although 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, WAS and X-CGD using retroviral vector-mediated gene transfer into CD34+ bone marrow cells. Although continued follow-up of all gene therapy patients will be required, data suggest that administration of retrovirally transduced mature T cells is a safe procedure. While 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. Experience at the NIH Clinical Center and other centers treating patients with advanced cancers with genetically engineered T cells modified using retroviral vectors indicates that these cells do not have a significant risk of malignant transformation in this setting.

Table 9.

number	protein[Homo sapiens]	expression	sequence	HLA-A11
	CT-RCC-1 HLA-A11-restricted HERV-E peptide peptide	clear cell renal carcinoma	ATFLGSKTWK	yes
1	serine-rich coiled-coil domain-containing protein 2	testis, heart, brain	kTFLGSKlpK	yes
2	F-box only protein 10	adrenal, skeletal muscle, testis, adrenal gland	qhYLaSKTWt	no
3	ufm1-specific protease 2	ectocervix, testis, ovary, kidney, stomach, intestine	ATFvGSrqWi	no
4	sodium/potassium/calcium exchanger 2	ovary, adrenal gland, blood, thyroid gland	iTFfGSiTWi	no
5	neuronal cell adhesion molecule	cerebellum, hypothalamus, cerebral cortex, neocortex	lTFqGSKThg	no
6	transmembrane protein 45A	melanoma cell lines, melanocytes	tcYLGSKTlf	no

BLAST search for peptides with at least 6 (or at least 5 identical + 1 conservative change) amino acids shared with CT-RCC-1 peptide sequence (ATFLGSKTWK). Capital/Underlined = amino acid identical to CT-RCC-1 peptide Capital/Not Underlined = conservative change.

In summary, this protocol is designed with a dose escalation design with the hope that off target toxicities related to TCR transduced T-cells will be unlikely to occur because 1) the TCR targets a foreign viral protein that are not expressed in healthy tissues, so there should be a low chance of toxicity from TCR transduced T-cells targeting healthy tissues that unexpectedly expresses the target, 2) the TCR came from a human who did not manifest any symptoms of autoimmunity 3) the TCR came from a human and therefore has been selected in thymus to not have autoreactivity, so autoimmunity is unlikely and 4) the TCR specificity has not been altered by affinity enhancement making it less likely that cross reactivity against normal human proteins has been introduced.

15.13 Risks in Relation to Benefit

Clinically, the approach is ethically acceptable because we are targeting a patient group with incurable metastatic cancers or hematological malignancies, which have become refractory to conventional therapy.

The risk of death due to complication from the protocol therapy is justified by the anticipated benefit of potentially eradicating the subject's underlying metastatic disease or hematologic malignancy.

16. INFORMED CONSENT PROCESSES AND PROCEDURES

Informed consent will be conducted following OHSRP Policy 301- Informed Consent.

An IRB-approved consent form will be provided to the participant electronically or by hard copy for review prior to consenting. The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved platforms). The investigational nature and objectives of this trial, the procedures, and their attendant risks and discomforts and potential benefits will be carefully explained to the participant in a private setting. The participant will be given as much time as they need to review the document and to consult with their family, friends, and personal health care providers. In addition, a study team member will be available to answer any questions.

A signed and dated informed consent document will be obtained by any investigator authorized to consent (See Key Study Personnel Page) prior to entry onto the study. Consent may be obtained with required signatures on the hard copy of the consent or on the electronic document.

When a document that is in electronic format is used for obtaining consent, this study may use the iMed platform which is 21 CFR, Part 11 compliant, to obtain the required signatures.

During the consent process, participants and investigators may view the same approved consent document simultaneously when participant is being consented in person at the Clinical Center or both may view individual copies of the approved consent document on screens in their respective locations remotely. Signatures may be obtained either by both directly signing on the device that the consenting investigator is using (when in person) or through iMed Mobile Signature Capture (remotely) which allows texting or emailing a link to the participant. That link allows the participant to review the consent, then proceed to sign on the device they are using.

Whether hard copy or electronic consent document, both the investigator and the participant will sign the document with a hand signature using a pen, finger, stylus, or mouse.

When done remotely, if the participant prefers to sign a hard copy, they may be instructed to sign and date the consent document during the discussion and mail, secure email or fax the signed document to the consenting investigator.

Whether in person or remotely, the privacy of the participant will be maintained.

Finally, the fully signed informed consent document will be stored in the electronic medical record, and the participant will receive a copy of the signed informed consent document.

At any time during participation in the protocol that new information becomes available relating to risks, adverse events, or toxicities, this information will be provided orally or in writing to all enrolled or prospective subject participants. Documentation will be provided to the IRB and if necessary, the informed consent amended to reflect relevant information.

A patent application on the HERV-E TCR sequence developed in our lab at the NHLBI was filed with the U.S Patent and Trademark Office. Because Dr. Childs is listed in the patent application, he will not be allowed to consent any subjects and will leave the room during the consent process.

17. PHARMACEUTICALS

T-CELL PRODUCT
Cell preparation (anti- HERV-E transduced PBL)

The procedure for expanding the human PBL and the certificate of analysis are similar to those approved by the food and drug administration and used at Loyola University Chicago in ongoing protocols evaluating cell therapy. The PBL will be transduced with retroviral supernatant containing the alpha chain and beta chain genes of the Anti-HERV-E. Note: Penicillin, Streptomycin, and gentamycin will not be used in the manufacture of products for patients.

Retroviral vector containing the anti-HERV-E gene

The retroviral vector supernatant encoding a T cell receptor directed against HERV-E was prepared and preserved following cGMP conditions in the Cellular Therapy Center at Loyola University Chicago. The batch of virus generated was cGMP qualified by Indiana University Vector Production Facility located in Indianapolis, IN.

The retroviral vector, HERV-E TCR CD34t 7G1, consists of 7,948 bps including the 5' LTR from the murine stem cell virus (promoter), packaging signal including the splicing donor (SD), splicing acceptor sites, alpha chain and beta chain genes of the anti-HERV-E TCR. The HERV-E TCR is also linked to a truncated CD34 chain (CD34t). We used PCR to link the α and β TCR chains together with a P2A self-cleavage sequence to facilitate high and equal expression of both TCR chains. The HERV-E TCR is linked to a CD34t by a different self-cleavage sequence (T2A) resulting in a final transgene cassette consisting of TCR α -TCR β- CD43t. This CD34t cassette lacks its intracellular signaling domain so it does not function in cells. The physical titer of the clinical vector will be measured in transducing units per mL following a titration on human PBL. The viral supernatant was aliquoted, snap frozen at –80 °C and is stored in a secure –80 °C freezer within the Cellular Therapy Center at Loyola University Chicago. There will be no re-use of the same unit of supernatant 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.

The SAMEN retroviral backbone can only produce infectious virus when introduced into a retroviral packaging cell line which contains the *gag*, *pol*, and *env* genes. The SAMEN vector containing the HERV-E TCR and CD34t cassette has been introduced into the PG13 retroviral producer cell line. PG13 uses the GALV envelope which has a host range of human and primate cells only. A PG13 clone was isolated that has high transduction efficiency. It was expanded in the GMP laboratory and a Master cell bank of 212 vials of 5 million cells per vial was established and cryopreserved. 6 liters of retroviral supernatant was generated from a vial from the Master cell bank and cryopreserved in 40 cc aliquots then frozen. The Master cell bank is stored in the liquid nitrogen freezer within the GMP facility and the cGMP qualified retroviral supernatant is stored in a locked -80ºC freezer also in the GMP facility. The virus titer or the retroviral supernatant was measured to be 7.4 x 10^5 TU/ml. The Master cell bank and retrovirus was sent in the Fall of 2016 to the Indiana University Vector Production Facility for GMP qualification (see attached COA for the Master cell bank or testing results for the virus). More specifically, 28 vials of Master cell bank and 822 cc of the virus supernatant (taken at the time of packaging) were sent to Indiana for GMP qualification.

CYCLOPHOSPHAMIDE

(Refer to FDA-approved package insert for complete product information)
Supply: Commercially available
Product Description: Cyclophosphamide is a nitrogen mustard-derivative alkylating agent. Following conversion to active metabolites in the liver, cyclophosphamide functions as an alkylating agent; the drug also possesses potent immunosuppressive activity. The serum half-life after IV administration ranges from

3 to 12 hours; the drug and/or its metabolites can be detected in the serum for up to 72 hours after administration.
Preparation: Reconstitute Cyclophosphamide with 0.9% Sodium Chloride Injection, USP only, using the volumes listed below in Table 10. Gently swirl the vial to dissolve the drug completely. Do not use Sterile Water for Injection, USP because it results in a hypotonic solution and should not be injected directly.
Storage and 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 to 8°C.
Administration: It will be diluted in 100 mL D5W and infused. The dose will be based on the patient's actual weight for all subjects except those who meet criteria for morbid obesity (i.e. BMI > 35); for morbidly obese subjects, cyclophosphamide dosing will be based on the practical weight. For definition of practical weight, refer to NHLBI Supportive Care Weight Based Dosing Guidelines.

Table 10.

Reconstitution for Direct Intravenous Injection		
Strength	**Volume of 0,9% Sodium Chloride**	**Cyclophosphamide concentration**
500 mg	25 mL	20 mg per ml
1 gram	50 mL	
2 grams	100 mL	

FLUDARABINE

(Refer to FDA-approved package insert for complete product information)
Supply: Commercially available
Product description: Fludarabine phosphate is commercially available as both a lyophilized powder for injection in vials containing 50 mg of fludarabine phosphate with mannitol 50 mg and sodium hydroxide for pH adjustment and a solution for injection in 2 mL vials containing 50 mg of fludarabine phosphate (25 mg/mL of fludarabine) with 25 mg/mL mannitol and sodium hydroxide for pH adjustment.
Preparation: Fludarabine lyophilized powder for injection should be reconstituted with 2 mL of sterile water
for injection, up to a concentration of 25 mg/mL. The prescribed dose of fludarabine should be diluted in 100 mL of either 0.9% sodium chloride or 5% dextrose in water for intravenous administration.
Storage and Stability: Fludarabine vials should be stored under refrigeration between 2-8° C (36-46°F). Reconstituted fludarabine phosphate is chemically and physically stable for 24 hours at room temperature or if refrigerated. The manufacturer recommends use of either the reconstituted powder for injection or the solution for injection (once diluted for administration) within 8 hours because neither product contains an antimicrobial preservative.
Administration: The prescribed dose of fludarabine should be diluted in 100 mL of either 0.9% sodium chloride or 5% dextrose in water for intravenous administration. The dose will be based on the patient's actual weight for all subjects except those who meet criteria for morbid obesity (i.e. BMI > 35); for morbidly obese subjects, fludarabine dosing will be based on the practical weight. For definition of practical weight, refer to NHLBI Supportive Care Weight Based Dosing Guidelines.

INTERLEUKIN-2 (Aldesleukin, Proleukin®, IL-2)

(Refer to FDA-approved package insert for complete product information)
Supply: Commercially available
Product description: Interleukin-2 is available as a parenteral formulation. It is supplied in individually boxed single-use vials. Each vial contains 22 x 10^6 IU (22 million IU) of interleukin-2 as a sterile, white to off-white 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).

Interleukin-2 vials contain a sterile, white to off-white, preservative free, lyophilized powder that requires reconstitution.
Preparation: Reconstitute each vial aseptically with 1.2 mL of sterile water for injection, USP. When reconstituted as directed, each milliliter (mL) contains 18 million IU of interleukin-2 (18 million IU per 1 mL). The resulting solution should be clear, colorless to slightly yellow liquid. The vial is for single-use only and any unused portion should be discarded. During reconstitution, the sterile water for injection, USP should be directed at the side of the vial and the contents gently swirled to avoid excess foaming. DO NOT SHAKE. Reconstitution with bacteriostatic water for injection, USP or 0.9% sodium chloride injection, USP should be avoided because of increased aggregation.
Storage and stability: Before and after reconstitution store in a refrigerator at 2º to 8º C (36^{O} to 46^{O} F). Do not freeze. Following reconstitution with sterile water for injection, USP, interleukin-2 reconstituted vials should be used within 24 hours.
Administration: The dosage will be calculated based on total body weight. The final dilution of IL-2 will be administered as an inpatient.

Note: None of the intravenous drugs used in this protocol will not be stored longer than 24 hours.

18. APPENDICES

A. ECOG Performance Status

GRADE	ECOG PERFORMANCE STATUS
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any selfcare; totally confined to bed or chair
5	Dead

B. Clinical management recommendations for IL-2 therapy

Issue	Considerations	Management
Venous Access	Central line (for possible vasopressors) Double or triple lumen Power inject and large volume capacity Minimize catheter associated infection	**Typical** PICC line placement Remove temporary lines at end of cycle **Variations** Broviac/Hickman catheter Subclavian/IJ catheter
IV Fluids	Maintenance of volume with CLS Boluses for blood pressure support Administration of drugs Replacement of electrolytes IL-2 only compatible with D5W	**Typical** D5NS or D5LR 10 ml – 125 ml/hr PRN KCL, HCO3, Mg replacement **Variations** D5W, NS, 0.45% NaCl
Infections	No active infections IV catheter likeliest source Avoid unnecessary in-dwelling catheters	**Typical** Prevention Gram + prophylactic antibiotic **Variations** Expanded coverage per hospital
Chills/rigors **Fever** **Constitutional symptoms**	Chills and rigors occur 1–2 hours after IL-2 Fever is common 2–4 hours after IL-2 Muscle joint aches continuous and progressive during IL-2 treatment	**Fever-Typical** Prophylaxis Acetaminophen 650 mg 30 min pre-dose, q 4–6 hrs and prn Indomethacin 25 mg q 6–8 hrs **Fever-Variation** Naproxen Ibuprofen **Chills-Typical** Meperidine 25 mg IV q 15 min PRN Morphine 2-4 mg IV q 15 min PRN
Nausea/vomiting	Episodic occurrence throughout therapy Nausea/Vomiting	**Typical-Prophylaxis** Ondansetron 0.15 mg/Kg q 8 hours **Variations** Granisetron 1 mg daily Ondansetron at longer interval

		Compazine 10 mg PO q 6 hours Use the antinausea agents PRN
Epigastric distress	Gastritis induced by stress, medications	**Typical** H2 blocker prophylaxis **Variation** PPI prophylaxis
Mucositis/stomatitis	Progressive with continued treatment	Typical No prophylaxis Oncology mouthwash
Diarrhea	Can be profuse and increase with therapy 5HT-3 antagonist anti-emetic prophylaxis may have positively impacted.	**Typical** Loperamide Lomotil (diphenoxylate hydrochloride and atropine sulfate) Narcotic (Opium Tincture) Break between IL-2 doses 5HT-3 antagonist prophylaxis
Patient monitoring	I&O, Weight Blood pressure, pulse, respirations, temperature Blood work EKG O2 saturation Mental status examination	Per shift and daily Q 2-4 hours Daily Continuous cardiac monitoring Q 2-4 hours Q 8 hours **Increase frequency as needed**
IL-2 dose and administration	- IL-2 incompatible with salt solutions - Dissolve in sterile water for injection. Dilute in 50 cc D5W - Stop infusion, flush IV tubing with 50 cc D5W before and after each dose.	
Hypotension	Maintain systolic BP 80-90 mmHg Blood pressure nadirs 4-6 hrs each dose with diminished recovery with cumulative dosing Prior to each dose anticipate ability to respond to next nadir Progressive refractoriness to support measures	Fluid boluses, 250-500 ml NS 2xday Increase maintenance fluid rate Phenylephrine 0.1-0.4 mcg/Kg/min Hold next dose Discontinue IL-2 **Variations:** Dopamine 1-6 μg/Kg/min Pressors with minimal fluids

		Fluids with pressors
Cardiac arrhythmias	**Sinus tachycardia** - Common and progresses over a cycle - Peaks 2-4 hours after dose with fever and hypotension - Must resolve prior to next dose **Troponin elevation** **Supraventricular tachycardia, atrial fibrillation** Less common **Atrial fibrillation** **Ventricular tachycardia**	Manage BP and fever Must stop Il- and will need an ECHO before next cycle of IL2 to rule out myocardial dysfunction. Future IL-2 may be considered if the ECHO is normal Medical Conversion Diltiazem as needed Digoxin Medical Conversion Acute treatment Discontinue IL-2
Renal function	**Oliguria** **Rising creatinine** **Urine output and creatinine resolve after discontinuation of IL-2** If only one kidney always consider obstruction of ureter	**Typical** Output less than 50-100 cc/8hours: Fluid bolus. If no improvement within 8 hours will hold IL-2 dose Consider to add Dopamine after 1-1.5 L of fluid boluses Creatinine >3-4 Stop NSAIDS and nephrotoxic antibiotics Hold overnight dose If morning creatinine improved continue **Variations** Dopamine 1-6 mcg/Kg/min Furosemide

Pulmonary	Tachypnea/Dyspnea Diagnose etiology and treat Hypoxic causes-Fluid overload, capillary leak, bronchospasm Non hypoxic causes Anxiety, fever, acidosis Maintain O2 sat > 92-95%	**Typical** Oxygen 2-4 L nasal cannula, increasing up to 35% rebreather Reassurance or sedative for anxiety, treat broncospasm or acidosis if appropriate Hold IL-2 dose if O2 sat < 95% **Variation** Furosemide Bronchodilators Monitor bicarbonate
Peripheral Edema	Expect to gain 5-10% body weight Treat edema symptomatically Entrapment of peripheral nerves in upper extremity may need therapy	Elevation, compression, limit fluid support in subsequent cycles Diuretics upon conclusion of IL-2 dosing are not necessary but may speed process Treat peripheral nerve pain
Neurotoxicity	Protean manifestations Gradual onset with sudden worsening near end of cycle May persist after cessation of therapy Delusions, Visual hallucinations	**Typical** Formal neurologic checks Enlist family evaluation Lorazepam and Haloperidol Hold IL-2 liberally for suspected neurotoxicity Warn patient of vivid dreams after discharge
Dermatologic	Rash, erythema, dry desquamation Pruritus Moist dermatitis	**Typical** Emollient lotions and creams Oatmeal bath Antihistamines Hold IL-2 dose **Variations** Gabapentin Naloxone Narcotics **Non-alcohol, no steroid topicals**
Metabolic	Hypomagnesemia, hypocalcemia (but low albumin – so corrected may be WNL), Hypokalemia.	Daily electrolyte panels

	Acidosis due to diarrhea, hypoperfusion Hypothyroidism a slow onset problem	Correct electrolytes cautiously prn Magnesium and HCO3, particularly if diarrhea a problem HCO3 < 18 meq/L hold dose of IL-2 Check TSH at beginning of cycle RL as support fluids may decrease need for HCO3
Hepatic	↑Bilirubin (up to 10) ↓Albumin (down to 1.8) ↑Hepatic aminotransferases	Monitor daily No intervention except if SGOT SGPT are >5x Resolves spontaneously Stop acetaminophen if bilirubin > 5
Hematologic	↓Platelets Lymphs ↓during IL-2, ↑post therapy Eosinophils progressively ↑with several cycles	Transfuse platelets if < 20,000/uL Other abnormalities require no intervention Significant anemia needs evaluation for cause
Endocrine	Hypothyroidism – slow onset after completion of treatment Requires serial monitoring	Check TFTs at beginning of cycle and monitor Thyroid function test with subsequent visits

Criteria for holding and stopping IL2 (see guidance below for when to delay or stop IL-2*)

System	Relative Criteria	Absolute Criteria	Supportive Measures
Cardiovascular	Sinus tachycardia 100–130 BPM (Persistent at the time of dosing after correcting hypotension, fever, and tachycardia and discontinuing dopamine)	Sinus tachycardia > 130 BPM ECG indications of ischemia Atrial fibrillation Supraventricular tachycardia Ventricular arrhythmias Elevated CKB-MB isoenzyme of troponin levels	Correction of fluid and electrolyte imbalances; chemical conversion or electrical conversion therapy.
Dermatologic	Moist desquamation, Rash, Erythema, Pruritus		Hydroxyzine 10-20mg po q6, prn; Diphenhydramine HCL 25-50mg, po, q4h, prn.

Gastrointestinal	Diarrhea, 1000 mL/8hours Ileus/abdominal distention Bilirubin > 7 mg/dL	Diarrhea 1000 mL/8 hours x 2 Vomiting not responsive to medication Severe abdominal distention affecting breathing Severe, unrelenting abdominal pain	Loperamide 2mg, po, q3h, prn; Diphenoxylate HCL 2.5mg and atropine sulfate 25mcg, po, q3h, prn.
Hemodynamic	Maximum Phenylephrine 1–1.5 mcg/kg/min Minimum Phenylephrine > 0.5 mcg/kg/min (To maintain acceptable BP and pulse criteria)	Maximum Phenylephrine 1.5-2.0 mcg/kg/min Minimum Phenylephrine > 0.8 mcg/kg/min	Fluid resuscitation Vasopressor support
Hemorrhagic	Sputum, emesis, or stool heme-positive Platelets 30,000-50,000/mm3	Frank blood in sputum, emesis, or stool Platelets < 30,000 mm^3	Transfusion with platelets
Infectious/Fever		Strong clinical suspicion or documented	Acetaminophen 650mg, po, q4-6h
Musculoskeletal	Weight gain > 15% Extreme tightness	Extreme paresthesias	Bedrest interspersed with activity. Diuretics prn.
Neurologic	Vivid dreams Mild anxiety Emotional lability	Hallucinations Persistent crying Mental status changes not reversible in 2 hours Unable to subtract serial “7 s” or spell “WORLD” backward Disorientation	Observation
Pulmonary	Resting shortness of breath 3–4 L O2 by nasal cannula for O2 saturation ≥ 95% Rales 1/3 up chest	> 4 L O2 by nasal cannula or 40% by mask to maintain ≥ 95% O2 saturation Endotracheal Intubation Moist rales 1/2 up chest Pleural effusion requiring tap or chest tube while on therapy	Stop Il-2 administration if ventilatory support is required.
Renal	Urine output 80–160 mL/8 hours Urine output 10-20 mL/hour	Urine < 80 mL/8 hours Urine output < 10 ml/hour Creatinine ≥ 3 mg/dL HCO3 ≤ 18 meq/dL	Fluid boluses or dopamine at renal doses. Dialysis for renal failure.

	Creatinine 2.5-2.9 mg/dL		

*** When to delay, or discontinue interleukin-2**

OBSERVATION CATEGORY	**ACTION**
Any relative criteria	Initiate corrective measure ± delay IL-2
≥ 3 relative criteria	Initiate corrective measures, delay IL-2 Stop IL-2 if not easily reversible
Any absolute criteria	Initiate corrective measure, delay IL-2 Stop IL-2 if not easily reversible

C. GUIDELINES FOR MANAGEMENT OF COMMON TOXICITIES THAT OCCUR AFTER T-CELL INFUSIONS

Infusions of TCRs T cells are rarely complicated by significant acute toxicities in the first 2 to 3 weeks after the infusion. In many cases the toxicities correlate with serum inflammatory cytokine levels.[144]

Note these are guidelines that might require modification based on clinical circumstances of each patient, and failure to exactly follow these guidelines is not a protocol deviation or violation.

For acute infusion reactions, the following guideline is recommended to be followed:

Acute Infusion Reactions PRN supportive care:

- Stop infusion
- If patient develops symptoms consistent with acute infusional, infusion of HERV-E TCR Transduced Autologous T Cells will be stopped.
- 0.9% Sodium Chloride Injection, by intravenous infusion at 500ml/hour, one time dose as needed for reaction with T-cell product. Infuse over 30 minutes. Give fluid bolus upon verbal order of provider (MD, NP or PA) for infusion reaction.
- Acetaminophen-325 mg tablet. Dose: 650mg (two tablets) by mouth one time dose as needed for febrile infusion reaction with HERV-E TCR Transduced Autologous T Cells. Given upon verbal order of provider (MD, NP or PA) for febrile infusion reaction.(Maximum adult acetaminophen daily dose: 3 grams/Day).
- Diphenhydramine Inj-50mg/ml. Dose: 50 mg, by intravenous push, one time dose as needed for PRN infusion reaction with HERV-E TCR Transduced Autologous T Cells.
- Hydrocortisone Inj. Dose 100 mg by intravenous push, one time dose as needed for sever infusion reaction (grade 4 infusion reaction by CTCAE 5.0 or when clinically indicated) with HERV-E TCR Transduced Autologous T Cells. Give upon verbal order of provider (MD, NP or PA) for infusion reaction.
- Albuterol Nebulization Solution-0.083% (2.5mg/3ml vial). Dose:2.5 mg by nebulizer one time dose as needed for infusion reaction with shortness of breath or wheezing with HERV-E TCR Transduced Autologous T Cells.
- Epinephrine Auto-Injection 0.3 mg (EpiPen). Dose 0.3 mg by intramuscular injection as needed for severe infusion reaction (grade 4 infusion reaction by CTCAE 5.0 or when clinically indicated) with HERV-E TCR Transduced Autologous T Cells. Give upon verbal order of provider (MD, NP or PA) for infusion reaction.
- HERV-E TCR Transduced Autologous T Cell infusion will be resumed at PI or AI discretion.

For all other reactions the following guidance is recommended to be followed (Note: Administration of corticosteroids should be avoided if at all possible to avoid killing or impairing the function of the infused TCR T-cells.

1. All patients with significant malignancy burdens and without a contradiction such as allergy should be started on allopurinol at the time of the start of the chemotherapy conditioning regimen or 1 day before the TCR T-cell infusion. The suggested allopurinol dose is 200 to 300 mg/day with a loading dose of 300 to 400 mg.
2. Vital signs should be checked a minimum of every 4 hours during hospitalization. Increasing the time interval between vital sign checks for patient convenience or other reasons should be avoided.
3. Strict ins and outs should be recorded on all patients.
4. As a minimum, keep hemoglobin greater than 8.0 g/dL and platelets greater than 20K/microliter.

5. Administer fresh frozen plasma (FFP) for a PTT 1.5-fold or more above the upper limit of normal.
6. For patients with an increased PTT, check the fibrinogen level and keep the fibrinogen level above 100 mg/dL with cryoprecipitate.
7. Fevers should be treated with acetaminophen and comfort measures. NSAIDs and corticosteroids should be avoided.
8. Patients with a heart rate persistently higher than 115/minute and fever should have vital signs checked every 2 hours.
9. Patients who are neutropenic and febrile should be receiving broad-spectrum antibiotics as per institutional guidelines
10. If the absolute neutrophil count becomes less than 500/microliter, Filgrastim or filgrastim-sndz may be initiated at a dose of 300 micrograms daily for patients under 70 kg in weight and a dose of 480 micrograms daily for patients over 70 kg in weight only in patients with absolute neutrophil counts less than 500/microliter. Filgrastim or filgrastim-sndz will be discontinued as soon as the absolute neutrophil count recovers to 1500/microliter.
11. Hypotension is a common toxicity requiring intensive care unit (ICU) admission. In general patients should be kept well-hydrated. Maintenance I.V. fluids (normal saline (NS) should be started on most patients with high fevers especially if oral intake is poor or the patient has tachycardia. I.V. fluids are not necessary for patients with good oral intake and mild fevers. For patients who are not having hypotension or tumor lysis syndrome, a generally even fluid balance should be strived for after allowing for insensible fluid losses in patients with high fevers. The baseline systolic blood pressure is defined for this protocol as the average of all systolic blood pressure readings obtained during the 24 hours prior to the TCR T-cell infusion. The first treatment for hypotension is administration of IV NS boluses.
    - Patients with a systolic blood pressure that is less than 80% of their baseline blood pressure and less than 100 mm Hg should receive a 1 L NS bolus.
    - Patients with a systolic blood pressure less than 85 mm Hg should receive a 1 L NS bolus regardless of baseline blood pressure.

These I.V. fluid management suggestions may need to be modified based on the clinical characteristics of individual patients such as pulmonary status, cardiac function, edema and other factors.

12. Patients receiving more than 1 fluid bolus for hypotension should have a stat EKG and troponin, and a cardiac echocardiogram within 24 hours of the second fluid bolus.
13. Patients should be transferred to the ICU under these circumstances. Patients not meeting these criteria could also require ICU admission at the discretion of the clinical team caring for the patient.
    - Systolic blood pressure less than 75% the patient's baseline blood pressure and less than 100 mm Hg after administration of a 1L NS bolus.
    - Anytime the systolic blood pressure is less than 90 mm Hg after a 1L NS bolus if 90 mm Hg is less than the patient's baseline systolic blood pressure.
    - Continuous tachycardia with a heart rate higher than 125 beats per minute on at least 2 occasions separated by 4 hours.
    - Oxygen requirement of more than a 4L standard nasal cannula
    - Greater than grade 2 neurological toxicity

14. All patients transferred to the ICU for hypotension or tachycardia should have a stat EKG and a cardiac echocardiogram within 24 hours of the time of transfer.
15. Patients with hypotension not responding to IV fluid resuscitation should be started on norepinephrine at doses called for by standard ICU guidelines.*
16. Patients should have a cardiac echocardiogram and an EKG within 12 hours of starting norepinephrine.

17. Patients in the ICU should get twice-daily labs (CBC with differential, acute care panel, mineral panel, hepatic panel, uric acid, LDH. Patients in the ICU should also get a daily troponin level).
18. Anecdotal evidence suggests that the IL-6 receptor blocker Tocilizumab can be an effective treatment for cytokine-release syndrome toxicities after TCR T-cell infusions. Tocilizumab may be administered under the following circumstances if the listed disorders are thought to be due to cytokine release from TCR T cells. Tocilizumab is administered at a dose of 4 mg/kg infused IV over 1 hour (dose should not exceed 800 mg).
    - Left ventricular ejection fraction less than 45% by echocardiogram
    - Creatinine greater than 2. 0- fold higher than the most recent level prior to TCR T-cell infusion
    - Norepinephrine requirement >3 micrograms/minute for 36 hours since the first administration of norepinephrine even if norepinephrine administration was not continuous.
    - Systolic blood pressure of 90 mm Hg cannot be maintained with norepinephrine.
    - Oxygen requirement 60% or greater fraction of inspired oxygen (FIO2) for more than 2 continuous hours.
    - Dyspnea that is severe enough to potentially require mechanical ventilation.
    - PTT or INR>2x upper limit of normal
    - Clinically-significant bleeding
    - Creatine kinase greater than 5x upper limit of normal for greater than 2 days 20.

*NOTE: In case of diagnosis of Cytokine Release Syndrome, we will consider high-dose of vasopressors if patient receives (all doses are required for ≥ 3 hours):[141]
- Norepinephrine monotherapy ≥ 20μg/Kg/min
- Dopamine monotherapy ≥ 10μg/Kg/min
- Phenylephrine monotherapy ≥ 200μg/Kg/min
- Epinephrine monotherapy ≥ 10μg/Kg/min
- If on vasopressin: Vasopressin + norepinephrine equivalent of ≥ 10μg/Kg/min
- If on combination vasopressors (not vasopressin): norepinephrine equivalent of ≥ 20μg/Kg/min

19. THERE IS NO EVIDENCE THAT TOCILIZUMAB HELPS NEUROLOGICAL TOXICITY, SO IT SHOULD NOT BE ADMINISTERED FOR THIS PURPOSE.
20. If no improvement in hypotension or tachycardia occurs within 6 hours of Tocilizumab infusion, consider other agents such as methylprednisolone 1 mg/kg every 12 hours or etanercept.
21. Avoid meperidine due to seizure risk.
22. All patients with grade 2 or greater neurological toxicities should get a neurology consult.
23. The following patients should receive dexamethasone 10 mg intravenously every 6 hours until the toxicities improve to Grade 1 or resolve or until at least 8 doses of dexamethasone have been given. Note: for seizures administer standard seizure therapies in addition to dexamethasone.
    1. Patients with Grade 3 or 4 neurological toxicities except that dexamethasone is not recommended for isolated Grade 3 headaches.
    2. Any generalized seizure.
24. In any patient with a cardiac left ventricular ejection fraction of 30% or less start methylprednisolone 100 mg IV every 6 hours.
25. In any patient likely to need mechanical ventilation within the next 4 hours, start methylprednisolone 100 mg IV every 6 hours.

D. For Cognitive Impaired subjects: Policy 403

REQUIREMENTS FOR THE DETERMINATION OF AN LAR'S APPROPRIATENESS TO CONSENT TO RESEARCH NOT INVOLVING GREATER THAN MINIMAL RISK (CATEGORY A) AND FOR RESEARCH INVOLVING GREATER THAN MINIMAL RISK BUT PRESENTING THE PROSPECT OF DIRECT BENEFIT TO THE INDIVIDUAL SUBJECTS (CATEGORY B)

First preference is #1. If not possible, go to option #2. If #2 is not possible, go to option #3.)

Cognitively Impaired Adults and Identification of a LAR	**Requirements for Determining Appropriateness of LAR to Consent to Research at Clinical Center (CC) and non- CC sites**	**Role of the LAR at all sites**
1. Adults who cannot consent and have a court-appointed guardian from a state that allows it[i] or a DPA[ii] for healthcare and/or research participation.	PI/designee[iii], unless the IRB designates an independent person(s) to perform this role (e.g., ACAT[iv] if the protocol is taking place at the CC), must assess appropriateness of LAR to consent to research. An appropriate LAR is one who at least: (a) Understands that the protocol involves research; (b) Understands the risks, potential benefits (if any), and alternatives to the study; and (c) Has sufficient reason to believe participation in the study is consistent with the subject's preferences and values.	LAR may give permission for the research and sign the consent form for the protocol on behalf of the subject.
2. Adults who cannot consent and who do not have a DPA or court-appointed guardian, but who are capable of understanding the DPA process and can assign a DPA[v].		
3. Adults who cannot consent, who do not have a DPA or court-appointed guardian, and cannot appoint a DPA: **At the CC**: A person at the highest level of the following may consent to research participation if found to be appropriate: 1) spouse or domestic partner[vi], 2) adult child, 3) parent, 4) sibling, 5) other close relative **At non-CC sites:** Consult with OGC to identify applicable state law.		

[i] A court appointed guardian may only consent to enroll a subject in research if the guardian has authority to do so under the laws of the state that issued the guardianship order and the terms of the guardianship order. The Office of the General Counsel (OGC) should be asked to review guardianship orders to determine if the guardian has legal authority to consent to the subject's participation in the research. PIs are encouraged to seek an OGC consultation in advance of a potential subject with a guardianship order coming to an NIH research site to enroll on a study.

[ii] DPA means the individual holding the durable power of attorney for healthcare. Consult with OGC if concerned about the authority provided in a DPA.

[iii] If the protocol is taking place at the CC, the PI's designee may be someone on the research team or a member of ACAT. If not at the CC, the PI's designee may be someone on the research team or an independent person outside of the research team if it is felt that the team does not have the required competencies to undertake the evaluation.

[iv] NIH Ability to Consent Team. For definition please see **Policy 403**.

E. Schedule of Events

SCREENING AND BASELINE ASSESSMENT

	Screening		Baseline
PROTOCOL TIMEPOINT	**1**		**2**
+/- days or months			
DAY			**<60 DAYS**
REGISTRATION			
Screening consent 97-H-0041	**X**		
Eligibility criteria	**X**		
Consent 18-H-0012			**X**
Durable Power of Attorney			**X**
High resolution HLA typing	**X**		
Confirmation of diagnosis of a clear cell renal cell carcinoma	**X**		
TREATMENT			
Recipient apheresis (venous access assessment)			**X**
Central line placement (3 Lumen IJ or PICC)			**O**
EVALUATIONS			
History and physical or progress note	**X**		**X**
ECOG	**X**		**X**
Vital signs (Temp, Pulse, BP, RR, Wt, O2 Sat)	**X**		**X**
Chemistry labs			
Acute care/mineral panels	**X**		
Hepatic Panel, Total Protein, LDH	**X**		
Prealbumin and lipid panel	**X**		**O**
Uric acid and CK	**X**		**O**
C-reactive protein			**X**
Hematology			
CBC with differential	**X**		**X**
PT, PTT,	**X**		**X**
Lymphocyte Phenotyping (TBNK, CD4)	**X**		**X**
DTM Blood Screening panel	**X**		
Antibody screen			
HIV	**X**		
HBV	**X**		
HVC	**X**		
Endocrine labs			
Thyroid panel (TSH, if TSH is abnormal, thenT4)			**X**
Cortisol Serum			**X**
Human Chorionic Gonadotropin/ Preg. test urine	**X**		**X**
Research blood/ biopsies			
Replication Competent Retrovirus Testing (1 EDTA tube with 4-5 ml). Ship to Indiana University Vector Production Facility			**X**
Tumor biopsy (Accessible lesions) up to 3 biopsies			**O**
Enumeration of absolute # of circulating CD34+/CD8+ T-cells [30 ml of blood collected in a green tube]. Only for patients screened for retreatment and a second apheresis is needed	**X**		
Pre-T cell product infusion Radiology tests			

Chest X-ray	**X**		
CT scan of the chest, abdomen and pelvis with IV contrast	**X**		**X**
MRI abdomen/pelvis with IV gadolinium*	**X**		
MRI brain with IV gadolinium	**X**		
CT of the brain with IV contrast	**O**		
CT neck with IV contrast	**O**		
PET scan (F-18 FDG PET-CT)			**X**
Bone scan (TC99 bone) and/or whole-body MRI	**O**		
Whole-body MRI			**O**
MRI for specific body area if metastasis present and clinically indicated			**O**
Pre-T cell product infusion tests			
Pulmonary function Test	**X**		
EKG	**X**		
2 D echo	**X**		**X**
Sesti Mibi			**O**
G6PD deficiency screen			**X**
24 hour creatinine clearance	**O**		
Type and Screen	**X**		
DAT			**X**
Pre-T cell product Infusion Consults			
DTM: vein assessment			**X**
Radiology Consult			**X**
X mandatory			
O Optional			
• **If patient cannot receive IV iodine contrast or for better characterization of metastatic lesions**			

TREATMENT PHASE

	Inpatient or Outpatient					Inpatient							
PROTOCOL TIMEPOINT	**3**	**4**	**5**	**6**		**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
+/- days or months													
DAY	**-4**	**-3**	**-2**	**-1**		**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
TREATMENT													
Central line placement (3 Lumen IJ or PICC)	**O**												
Immunoablative regimen													
Cyclophosphamide 1000 mg/m2	**X**												
Fludarabine 30mg/m2	**X**	**X**	**X**										
Admission to the inpatient ward				**O**									
HERV-E TCR transduced CD8+/CD34+ enriched T cells						**X**							
Supportive Medications													
IL2 2 million U/M2 q 12 IV**						**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
PCP and Toxo prophylaxis (Bactrim or others)	**X**												
Antifungal (Fluconazole or others)						**O**							
Antiviral prophylaxis (Acyclovir or others)	**X**												
Filgrastim						**O**	**O**	**O**	**O**	**O**	**O**	**O**	**O**
EVALUATIONS													
History and physical or progress note						**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
ECOG	**O**					**O**							**O**
Vital signs (Temp, Pulse, BP, RR, Wt, O2 Sat)	**X**	**X**	**X**			**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Chemistry labs													
Acute care/mineral panels	**X**					**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Hepatic Panel, Total Protein, LDH	**X**					**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Quantitative immunoglobulins (IGG, IGA, IGM)													
Uric acid and CK	**O**					**X**	**O**	**O**	**O**	**O**	**O**	**O**	**O**
C-reactive protein	**X**					**X**		**X**		**X**			**X**
Hematology													
CBC with differential	**X**					**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**
PT, PTT	**X**					**X**	**x**	**x**	**x**	**x**	**x**	**x**	**x**
Lymphocyte Phenotyping (TBNK, CD4)						**X**		**X**		**X**		**x**	
Type and Screen	**O**	**O**				**X**				**X**			
Endocrine Labs													
Thyroid panel (TSH, if TSH is abnormal, thenT4) and antithyroid panel only if prior abnormal as dictated by PI (THY2).						**x**							**x**
Research blood/ biopsies													
Enumeration of absolute # of circulating HERV-E TCR T-cells [30 ml of blood collected in a green tube]	**X**					**X****		**X**		**X**			**X**
Cytokine studies (1 SST Red Serum tube (10cc) tubes to order)	**X**					**X****		**x**		**x**			**x**
Tumor biopsy up to 3 biopsies							**O**	**O**	**O**	**O**	**O**	**O**	**O**
X mandatory													
O Optional													
*** patient can be discharged after completion of IL-2 if clinically stable.**													

**** Prior to chemotherapy and prior to T-cell infusion respectively**																			

FOLLOW-UP

		INPATIENT or OUTPATIENT								OUTPATIENT								
PROTOCOL TIMEPOINT		15	16	17	18	19	20	21		22	23	24	25	26	27	28	29	30
+/- days or months								+/- 2		+/- 3	+/- 5	+/- 7	+/- 7	+/- 7	+/- 14	+/- 14	+/- 30	+/- 30
DAY		8	9	10	11	12	13	14		21	30	60	90	120	180	240	300	360
EVALUATIONS																		
History and physical or progress note		O	O	O	O	O	O	X		X	X	X	X	X	X	X	X	X
ECOG								X		X	X	X	X	X	X	X	X	X
Vital signs (Temp, Pulse, BP, RR, Wt, O2 Sat)		O	O	O	O	O	O	X		X	X	X	X	X	X	X	X	X
Chemistry labs																		
Acute care/mineral panels		O	O	O	O	O	O	X		x	x	x	x	x	x	x	x	x
Hepatic Panel, Total Protein, LDH		O	O	O	O	O	O	X		x	x	x	x	x	x	x	x	x
Prealbumin and lipid panel								O		O	O	O	O	O	O	O	O	O
Quantitative immunoglobulins (IGG, IGA, IGM)										X	O	O	O	O	O	O	O	O
Uric acid and CK		O	O	O	O	O	O	X										
C-reactive protein								X		X	X	X	X	X	X	X	X	X
Hematology																		
CBC with differential		O	O	O	O	O	O	X		X	X	X	X	X	X	X	X	X
PT, PTT,		O	O	O	O	O	O	X		X	X	X	X	X	X	X	X	X
Lymphocyte Phenotyping (TBNK, CD4)								X		X	X	X	X	X	X	X	X	X
Endocrine labs																		
Thyroid panel (TSH, if TSH is abnormal, thenT4) and antithyroid panel (THY2)								X		O	O	O	O	O	O	O	O	O
Research blood/ biopsies																		
Enumeration of absolute number of circulating HERV-E TCR transduced CD8+/CD34+ enriched T-cells [30 ml of peripheral blood collected in a green to tubes]		O	O	O	O	O	O	X		X	X	X	X	X	X	X	X	X
Cytokine studies (1 SST Red Serum tube (10cc) tubes to order)		O	O	O	O	O	O	X		X	X	X	X	X	X	X	X	X
Replication Competent Retrovirus Testing (1 EDTA tube with 4-5 ml**). Ship to Indiana University Vector Production Facility**													X		X			X
Tumor biopsy (Accessible lesions) up to 3 biopsies		O	O	O	O	O	O	O		O	O	O	O	O	O	O	O	O
Post-T cell product infusion Radiology tests																		
CT scan of the abdomen and pelvis with IV contrast											X	O[t]	X	X	X	X	X	X
MRI abdomen/pelvis with IV gadolinium**											X	O	X	X	X	X	X	X
MRI brain with IV gadolinium[#]											X	X	X	X	X	X	X	X
CT of the brain with IV contrast[#]											X	X	X	X	X	X	X	X

CT neck with IV contrast[#]											**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
PET scan (F-18 FDG PET-CT)												**X**		**X**	**X**	**X**	**X**	**X**
Bone scan (TC99 bone)[#]											**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Whole-body MRI[#]											**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
MRI for specific body area if metastasis present and clinically indicated											**O**	**O**	**O**	**O**	**O**	**O**	**O**	**O**
Clarifications																		
X mandatory																		
O Optional																		
*** patient can be discharged after completion of IL-2 if clinically stable.** **** If patient cannot receive IV iodine contrast or for better characterization of metastatic lesions** **[t] Scan only needed if progression found at D+30. If disease progression is confirmed, no additional scans are required for that subject.** **[#] Perform only when baseline imaging test positive for metastatic disease**																		

F. Interleukin-15 (NSC 745101)

Classification: Recombinant human interleukin-15 (rhIL-15) is a cytokine of the 4-alpha helix bundle family of cytokines whose mature form consists of 115 amino acids. It has two cysteine disulfide cross linkages at positions Cys 42-Cys 88 and Cys 35-Cys 85.

M.W.: 12,898.8 Daltons

Mode of Action: IL-15 interacts with a private receptor subunit IL- 15R alpha as well as the IL-2/IL-15R beta chain shared with IL-2 and the common gamma chain shared with IL-2, IL-4, IL-7, IL-9 and IL-21. IL-15 shares a number of biological activities with IL-2, including stimulation of the proliferation of activated CD4+, CD8+ as well as gamma-delta subsets of T cells. IL-15 also stimulates the proliferation of NK cells and acts as a co-stimulator with IL-12 to facilitate the production of Interferon-gamma and TNF-alpha.

How Supplied: IL-15 is supplied by the Pharmaceutical Management Branch, DCTD, NCI as a sterile, frozen liquid product in single use vials containing no preservatives. IL-15 is supplied as a 0.49 mg/mL, 0.3 mL vial formulated in 25 mM sodium phosphate containing 0.5 M sodium chloride at a pH of 7.4.

IL-15 supply will be provided by the NCI to Michael I. Nishimura, Loyola University, Chicago for the purposes of improving HERV-E TCR transduction of T cells according to applicable procedures.

Preparation: Vials of frozen IL-15 should be thawed at ambient room temperature and used within 5 hours of thawing. Upon thawing, the solution should be clear and colorless with no evidence of particulates or foreign matter.

Storage:IL-15 vials should be stored at or below (– 70°C).

If a storage temperature excursion is identified, promptly return IL-15 vials to – 70°C and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to PMBAfterHours@mail.nih.gov for determination of suitability.

Stability: Stability studies are ongoing.

Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Drug Accountability Record Form (DARF).

19. REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, et al: Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 136:E359-86, 2015
2. Shuch B, Amin A, Armstrong AJ, et al: Understanding pathologic variants of renal cell carcinoma: distilling therapeutic opportunities from biologic complexity. Eur Urol 67:85-97, 2015
3. Riazalhosseini Y, Lathrop M: Precision medicine from the renal cancer genome. Nat Rev Nephrol 12:655-666, 2016
4. Latif F, Tory K, Gnarra J, et al: Identification of the von Hippel-Lindau disease tumor suppressor gene. Science 260:1317-20, 1993
5. Gnarra JR, Tory K, Weng Y, et al: Mutations of the VHL tumour suppressor gene in renal carcinoma. Nat Genet 7:85-90, 1994
6. Banks RE, Tirukonda P, Taylor C, et al: Genetic and epigenetic analysis of von Hippel-Lindau (VHL) gene alterations and relationship with clinical variables in sporadic renal cancer. Cancer Res 66:2000-11, 2006
7. Moore LE, Nickerson ML, Brennan P, et al: Von Hippel-Lindau (VHL) inactivation in sporadic clear cell renal cancer: associations with germline VHL polymorphisms and etiologic risk factors. PLoS Genet 7:e1002312, 2011
8. Land SC, Tee AR: Hypoxia-inducible factor 1alpha is regulated by the mammalian target of rapamycin (mTOR) via an mTOR signaling motif. J Biol Chem 282:20534-43, 2007
9. Velickovic M, Delahunt B, McIver B, et al: Intragenic PTEN/MMAC1 loss of heterozygosity in conventional (clear-cell) renal cell carcinoma is associated with poor patient prognosis. Mod Pathol 15:479-85, 2002
10. Shin Lee J, Seok Kim H, Bok Kim Y, et al: Expression of PTEN in renal cell carcinoma and its relation to tumor behavior and growth. J Surg Oncol 84:166-72, 2003
11. Horiguchi A, Oya M, Uchida A, et al: Elevated Akt activation and its impact on clinicopathological features of renal cell carcinoma. J Urol 169:710-3, 2003
12. Cancer Genome Atlas Research N: Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature 499:43-9, 2013
13. Sato Y, Yoshizato T, Shiraishi Y, et al: Integrated molecular analysis of clear-cell renal cell carcinoma. Nat Genet 45:860-7, 2013
14. Scelo G, Riazalhosseini Y, Greger L, et al: Variation in genomic landscape of clear cell renal cell carcinoma across Europe. Nat Commun 5:5135, 2014
15. Varela I, Tarpey P, Raine K, et al: Exome sequencing identifies frequent mutation of the SWI/SNF complex gene PBRM1 in renal carcinoma. Nature 469:539-42, 2011
16. Dalgliesh GL, Furge K, Greenman C, et al: Systematic sequencing of renal carcinoma reveals inactivation of histone modifying genes. Nature 463:360-3, 2010
17. van Haaften G, Dalgliesh GL, Davies H, et al: Somatic mutations of the histone H3K27 demethylase gene UTX in human cancer. Nat Genet 41:521-3, 2009

18. Shuch B, Said J, La Rochelle JC, et al: Cytoreductive nephrectomy for kidney cancer with sarcomatoid histology--is up-front resection indicated and, if not, is it avoidable? J Urol 182:2164-71, 2009
19. Shuch B, Bratslavsky G, Shih J, et al: Impact of pathological tumour characteristics in patients with sarcomatoid renal cell carcinoma. BJU Int 109:1600-6, 2012
20. Cheville JC, Lohse CM, Zincke H, et al: Sarcomatoid renal cell carcinoma: an examination of underlying histologic subtype and an analysis of associations with patient outcome. Am J Surg Pathol 28:435-41, 2004
21. Shuch B, Bratslavsky G, Linehan WM, et al: Sarcomatoid renal cell carcinoma: a comprehensive review of the biology and current treatment strategies. Oncologist 17:46-54, 2012
22. Malouf GG, Ali SM, Wang K, et al: Genomic Characterization of Renal Cell Carcinoma with Sarcomatoid Dedifferentiation Pinpoints Recurrent Genomic Alterations. Eur Urol 70:348-57, 2016
23. Bi M, Zhao S, Said JW, et al: Genomic characterization of sarcomatoid transformation in clear cell renal cell carcinoma. Proc Natl Acad Sci U S A 113:2170-5, 2016
24. Spontaneous regression of cancer. Lancet 2:896, 1966
25. Everson TC: Spontaneous regression of cancer. Prog Clin Cancer 3:79-95, 1967
26. Fyfe G, Fisher RI, Rosenberg SA, et al: Results of treatment of 255 patients with metastatic renal cell carcinoma who received high-dose recombinant interleukin-2 therapy. J Clin Oncol 13:688-96, 1995
27. Fisher RI, Rosenberg SA, Fyfe G: Long-term survival update for high-dose recombinant interleukin-2 in patients with renal cell carcinoma. Cancer J Sci Am 6 Suppl 1:S55-7, 2000
28. Choueiri TK, Motzer RJ: Systemic Therapy for Metastatic Renal-Cell Carcinoma. N Engl J Med 376:354-366, 2017
29. Keir ME, Francisco LM, Sharpe AH: PD-1 and its ligands in T-cell immunity. Curr Opin Immunol 19:309-14, 2007
30. Massari F, Santoni M, Ciccarese C, et al: PD-1 blockade therapy in renal cell carcinoma: current studies and future promises. Cancer Treat Rev 41:114-21, 2015
31. Motzer RJ, Escudier B, McDermott DF, et al: Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma. N Engl J Med 373:1803-13, 2015
32. Childs RW, Clave E, Tisdale J, et al: Successful treatment of metastatic renal cell carcinoma with a nonmyeloablative allogeneic peripheral-blood progenitor-cell transplant: evidence for a graft-versus-tumor effect. J Clin Oncol 17:2044-9, 1999
33. Childs R, Chernoff A, Contentin N, et al: Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. N Engl J Med 343:750-8, 2000
34. Ueno NT, Cheng YC: The future of allogeneic hematopoietic stem cell transplantation for metastatic renal cell carcinoma in the era of target-specific therapy. Bone Marrow Transplant 38:711-4, 2006
35. Dudley ME, Yang JC, Sherry R, et al: Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. J Clin Oncol 26:5233-9, 2008

36. Kawakami Y, Eliyahu S, Jennings C, et al: Recognition of multiple epitopes in the human melanoma antigen gp100 by tumor-infiltrating T lymphocytes associated with in vivo tumor regression. J Immunol 154:3961-8, 1995

37. Restifo NP, Dudley ME, Rosenberg SA: Adoptive immunotherapy for cancer: harnessing the T cell response. Nat Rev Immunol 12:269-81, 2012

38. Stevanovic S, Draper LM, Langhan MM, et al: Complete regression of metastatic cervical cancer after treatment with human papillomavirus-targeted tumor-infiltrating T cells. J Clin Oncol 33:1543-50, 2015

39. Anders K, Buschow C, Herrmann A, et al: Oncogene-targeting T cells reject large tumors while oncogene inactivation selects escape variants in mouse models of cancer. Cancer Cell 20:755-67, 2011

40. Robbins PF, Morgan RA, Feldman SA, et al: Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. J Clin Oncol 29:917-24, 2011

41. Parkhurst MR, Yang JC, Langan RC, et al: T Cells Targeting Carcinoembryonic Antigen Can Mediate Regression of Metastatic Colorectal Cancer but Induce Severe Transient Colitis. Mol Ther 19:620-626, 2011

42. Johnson LA, Morgan RA, Dudley ME, et al: Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. Blood 114:535-46, 2009

43. Chodon T, Comin-Anduix B, Chmielowski B, et al: Adoptive transfer of MART-1 T-cell receptor transgenic lymphocytes and dendritic cell vaccination in patients with metastatic melanoma. Clin Cancer Res 20:2457-65, 2014

44. Morgan RA, Chinnasamy N, Abate-Daga D, et al: Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy. J Immunother 36:133-51, 2013

45. Marusyk A, Polyak K: Tumor heterogeneity: causes and consequences. Biochim Biophys Acta 1805:105-17, 2010

46. Fidler IJ, Hart IR: Biological diversity in metastatic neoplasms: origins and implications. Science 217:998-1003, 1982

47. Beatty GL, Haas AR, Maus MV, et al: Mesothelin-specific chimeric antigen receptor mRNA-engineered T cells induce anti-tumor activity in solid malignancies. Cancer Immunol Res 2:112-20, 2014

48. Adusumilli PS, Cherkassky L, Villena-Vargas J, et al: Regional delivery of mesothelin-targeted CAR T cell therapy generates potent and long-lasting CD4-dependent tumor immunity. Sci Transl Med 6:261ra151, 2014

49. Beatty GLea: Safety and antitumor activity of chimeric antigen receptor modified T cells in patients with chemotherapy refractory metastatic pancreatic cancer [abstract]. J. Clin. Oncol. 33 (Suppl.), 3007 2015

50. Tanyi Jea: Safety and feasibility of chimeric antigen receptor modified T cells directed against mesothelin (CART-meso) in patients with mesothelin expressing cancers [abstract]. Cancer Res. 75 (Suppl.), CT105 2015

51. Louis CU, Savoldo B, Dotti G, et al: Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. Blood 118:6050-6, 2011

52. Singh N, Liu X, Hulitt J, et al: Nature of tumor control by permanently and transiently modified GD2 chimeric antigen receptor T cells in xenograft models of neuroblastoma. Cancer Immunol Res 2:1059-70, 2014

53. Johnson LA, Scholler J, Ohkuri T, et al: Rational development and characterization of humanized anti-EGFR variant III chimeric antigen receptor T cells for glioblastoma. Sci Transl Med 7:275ra22, 2015

54. Koneru M, Purdon TJ, Spriggs D, et al: IL-12 secreting tumor-targeted chimeric antigen receptor T cells eradicate ovarian tumors in vivo. Oncoimmunology 4:e994446, 2015

55. Hanley PJ, Cruz CR, Shpall EJ, et al: Improving clinical outcomes using adoptively transferred immune cells from umbilical cord blood. Cytotherapy 12:713-20, 2010

56. Apperley JF, Jones L, Hale G, et al: Bone marrow transplantation for patients with chronic myeloid leukaemia: T-cell depletion with Campath-1 reduces the incidence of graft-versus-host disease but may increase the risk of leukaemic relapse. Bone Marrow Transplant 1:53-66, 1986

57. Mitsuyasu RT, Champlin RE, Gale RP, et al: Treatment of donor bone marrow with monoclonal anti-T-cell antibody and complement for the prevention of graft-versus-host disease. A prospective, randomized, double-blind trial. Ann Intern Med 105:20-6, 1986

58. Drobyski WR, Keever CA, Roth MS, et al: Salvage immunotherapy using donor leukocyte infusions as treatment for relapsed chronic myelogenous leukemia after allogeneic bone marrow transplantation: efficacy and toxicity of a defined T-cell dose. Blood 82:2310-8, 1993

59. Collins RH, Jr., Shpilberg O, Drobyski WR, et al: Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. J Clin Oncol 15:433-44, 1997

60. Dudley ME, Wunderlich JR, Robbins PF, et al: Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science 298:850-4, 2002

61. Yee C, Thompson JA, Roche P, et al: Melanocyte destruction after antigen-specific immunotherapy of melanoma: direct evidence of t cell-mediated vitiligo. J Exp Med 192:1637-44, 2000

62. Kochenderfer JN, Dudley ME, Feldman SA, et al: B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. Blood 119:2709-20, 2012

63. Kochenderfer JN, Rosenberg SA: Treating B-cell cancer with T cells expressing anti-CD19 chimeric antigen receptors. Nat Rev Clin Oncol 10:267-76, 2013

64. Morgan RA, Yang JC, Kitano M, et al: Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. Mol Ther 18:843-51, 2010

65. Lamers CH, Sleijfer S, van Steenbergen S, et al: Treatment of metastatic renal cell carcinoma with CAIX CAR-engineered T cells: clinical evaluation and management of on-target toxicity. Mol Ther 21:904-12, 2013

66. Lamers CH, Sleijfer S, Vulto AG, et al: Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience. J Clin Oncol 24:e20-2, 2006

67. Grupp SA, Kalos M, Barrett D, et al: Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med 368:1509-18, 2013
68. Brentjens RJ, Davila ML, Riviere I, et al: CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. Sci Transl Med 5:177ra38, 2013
69. Davila ML, Riviere I, Wang X, et al: Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Sci Transl Med 6:224ra25, 2014
70. Weber JS, Kahler KC, Hauschild A: Management of immune-related adverse events and kinetics of response with ipilimumab. J Clin Oncol 30:2691-7, 2012
71. Wolchok JD, Neyns B, Linette G, et al: Ipilimumab monotherapy in patients with pretreated advanced melanoma: a randomised, double-blind, multicentre, phase 2, dose-ranging study. Lancet Oncol 11:155-64, 2010
72. Brentjens RJ, Riviere I, Park JH, et al: Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. Blood 118:4817-28, 2011
73. Linette GP, Stadtmauer EA, Maus MV, et al: Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. Blood 122:863-71, 2013
74. Cameron BJ, Gerry AB, Dukes J, et al: Identification of a Titin-derived HLA-A1-presented peptide as a cross-reactive target for engineered MAGE A3-directed T cells. Sci Transl Med 5:197ra103, 2013
75. van Loenen MM, de Boer R, Amir AL, et al: Mixed T cell receptor dimers harbor potentially harmful neoreactivity. Proc Natl Acad Sci U S A 107:10972-7, 2010
76. Bendle GM, Linnemann C, Hooijkaas AI, et al: Lethal graft-versus-host disease in mouse models of T cell receptor gene therapy. Nat Med 16:565-70, 1p following 570, 2010
77. Rosenberg SA: Of mice, not men: no evidence for graft-versus-host disease in humans receiving T-cell receptor-transduced autologous T cells. Mol Ther 18:1744-5, 2010
78. Ahmed N, Brawley VS, Hegde M, et al: Human Epidermal Growth Factor Receptor 2 (HER2) -Specific Chimeric Antigen Receptor-Modified T Cells for the Immunotherapy of HER2-Positive Sarcoma. J Clin Oncol 33:1688-96, 2015
79. Hacein-Bey-Abina S, Garrigue A, Wang GP, et al: Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. J Clin Invest 118:3132-42, 2008
80. Howe SJ, Mansour MR, Schwarzwaelder K, et al: Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. J Clin Invest 118:3143-50, 2008
81. Stein S, Ott MG, Schultze-Strasser S, et al: Genomic instability and myelodysplasia with monosomy 7 consequent to EVI1 activation after gene therapy for chronic granulomatous disease. Nat Med 16:198-204, 2010
82. Braun CJ, Boztug K, Paruzynski A, et al: Gene therapy for Wiskott-Aldrich syndrome--long-term efficacy and genotoxicity. Sci Transl Med 6:227ra33, 2014
83. Cavazzana-Calvo M, Payen E, Negre O, et al: Transfusion independence and HMGA2 activation after gene therapy of human beta-thalassaemia. Nature 467:318-22, 2010
84. J. Joseph Melenhorst ea: TET2 Gene Disruption via Lentiviral Integration Promotes CAR T Cell Expansion and
Persistence. American Society Gene Cell Therapy, 2017

85. Younan PM, Polacino P, Kowalski JP, et al: Positive selection of mC46-expressing CD4+ T cells and maintenance of virus specific immunity in a primate AIDS model. Blood 122:179-87, 2013
86. D'Souza V, Summers MF: How retroviruses select their genomes. Nat Rev Microbiol 3:643-55, 2005
87. Griffiths DJ: Endogenous retroviruses in the human genome sequence. Genome Biol 2:REVIEWS1017, 2001
88. Mayer J, Blomberg J, Seal RL: A revised nomenclature for transcribed human endogenous retroviral loci. Mob DNA 2:7, 2011
89. Young GR, Eksmond U, Salcedo R, et al: Resurrection of endogenous retroviruses in antibody-deficient mice. Nature 491:774-8, 2012
90. Yu P, Lubben W, Slomka H, et al: Nucleic acid-sensing Toll-like receptors are essential for the control of endogenous retrovirus viremia and ERV-induced tumors. Immunity 37:867-79, 2012
91. Muster T, Waltenberger A, Grassauer A, et al: An endogenous retrovirus derived from human melanoma cells. Cancer Res 63:8735-41, 2003
92. Buscher K, Hahn S, Hofmann M, et al: Expression of the human endogenous retrovirus-K transmembrane envelope, Rec and Np9 proteins in melanomas and melanoma cell lines. Melanoma Res 16:223-34, 2006
93. Wang-Johanning F, Frost AR, Jian B, et al: Quantitation of HERV-K env gene expression and splicing in human breast cancer. Oncogene 22:1528-35, 2003
94. Wang-Johanning F, Frost AR, Jian B, et al: Detecting the expression of human endogenous retrovirus E envelope transcripts in human prostate adenocarcinoma. Cancer 98:187-97, 2003
95. Menendez L, Benigno BB, McDonald JF: L1 and HERV-W retrotransposons are hypomethylated in human ovarian carcinomas. Mol Cancer 3:12, 2004
96. Takahashi Y, Harashima N, Kajigaya S, et al: Regression of human kidney cancer following allogeneic stem cell transplantation is associated with recognition of an HERV-E antigen by T cells. J Clin Invest 118:1099-109, 2008
97. Cherkasova E, Malinzak E, Rao S, et al: Inactivation of the von Hippel-Lindau tumor suppressor leads to selective expression of a human endogenous retrovirus in kidney cancer. Oncogene 30:4697-706, 2011
98. Rooney MS, Shukla SA, Wu CJ, et al: Molecular and genetic properties of tumors associated with local immune cytolytic activity. Cell 160:48-61, 2015
99. Cherkasova E, Scrivani C, Doh S, et al: Detection of an Immunogenic HERV-E Envelope with Selective Expression in Clear Cell Kidney Cancer. Cancer Res 76:2177-85, 2016
100. Szpakowski S, Sun X, Lage JM, et al: Loss of epigenetic silencing in tumors preferentially affects primate-specific retroelements. Gene 448:151-67, 2009
101. Kassiotis G, Stoye JP: Immune responses to endogenous retroelements: taking the bad with the good. Nat Rev Immunol 16:207-19, 2016
102. Young AC, Craven RA, Cohen D, et al: Analysis of VHL Gene Alterations and their Relationship to Clinical Parameters in Sporadic Conventional Renal Cell Carcinoma. Clin Cancer Res 15:7582-7592, 2009

103. Clay TM, Custer MC, Sachs J, et al: Efficient transfer of a tumor antigen-reactive TCR to human peripheral blood lymphocytes confers anti-tumor reactivity. J Immunol 163:507-13, 1999

104. Roszkowski JJ, Yu DC, Rubinstein MP, et al: CD8-independent tumor cell recognition is a property of the T cell receptor and not the T cell. J Immunol 170:2582-9, 2003

105. Lam JS, Reeves ME, Cowherd R, et al: Improved gene transfer into human lymphocytes using retroviruses with the gibbon ape leukemia virus envelope. Hum Gene Ther 7:1415-22, 1996

106. Roszkowski JJ, Lyons GE, Kast WM, et al: Simultaneous generation of CD8+ and CD4+ melanoma-reactive T cells by retroviral-mediated transfer of a single T-cell receptor. Cancer Res 65:1570-6, 2005

107. Rapoport AP, Stadtmauer EA, Binder-Scholl GK, et al: NY-ESO-1-specific TCR-engineered T cells mediate sustained antigen-specific antitumor effects in myeloma. Nat Med 21:914-21, 2015

108. Miller AD, Garcia JV, von Suhr N, et al: Construction and properties of retrovirus packaging cells based on gibbon ape leukemia virus. J Virol 65:2220-4, 1991

109. Hollenbach JA, Thomson G, Cao K, et al: HLA diversity, differentiation, and haplotype evolution in Mesoamerican Natives. Hum Immunol 62:378-90, 2001

110. Gattinoni L, Finkelstein SE, Klebanoff CA, et al: Removal of homeostatic cytokine sinks by lymphodepletion enhances the efficacy of adoptively transferred tumor-specific CD8+ T cells. J Exp Med 202:907-12, 2005

111. North RJ: Cyclophosphamide-facilitated adoptive immunotherapy of an established tumor depends on elimination of tumor-induced suppressor T cells. J Exp Med 155:1063-74, 1982

112. Muranski P, Boni A, Wrzesinski C, et al: Increased intensity lymphodepletion and adoptive immunotherapy--how far can we go? Nat Clin Pract Oncol 3:668-81, 2006

113. Klebanoff CA, Khong HT, Antony PA, et al: Sinks, suppressors and antigen presenters: how lymphodepletion enhances T cell-mediated tumor immunotherapy. Trends Immunol 26:111-7, 2005

114. Rosenberg SA: Cell transfer immunotherapy for metastatic solid cancer--what clinicians need to know. Nat Rev Clin Oncol 8:577-85, 2011

115. Rosenberg SA, Yang JC, Schwartzentruber DJ, et al: Recombinant fowlpox viruses encoding the anchor-modified gp100 melanoma antigen can generate antitumor immune responses in patients with metastatic melanoma. Clin Cancer Res 9:2973-80, 2003

116. Gattinoni L, Powell DJ, Jr., Rosenberg SA, et al: Adoptive immunotherapy for cancer: building on success. Nat Rev Immunol 6:383-93, 2006

117. Dumitru CA, Moses K, Trellakis S, et al: Neutrophils and granulocytic myeloid-derived suppressor cells: immunophenotyping, cell biology and clinical relevance in human oncology. Cancer Immunol Immunother 61:1155-67, 2012

118. Paulos CM, Wrzesinski C, Kaiser A, et al: Microbial translocation augments the function of adoptively transferred self/tumor-specific CD8+ T cells via TLR4 signaling. J Clin Invest 117:2197-204, 2007

119. Goff SL, Dudley ME, Citrin DE, et al: Randomized, Prospective Evaluation Comparing Intensity of Lymphodepletion Before Adoptive Transfer of Tumor-Infiltrating Lymphocytes for Patients With Metastatic Melanoma. J Clin Oncol 34:2389-97, 2016

120. Tran E, Robbins PF, Lu YC, et al: T-Cell Transfer Therapy Targeting Mutant KRAS in Cancer. N Engl J Med 375:2255-2262, 2016

121. Li S, Yang J, Urban FA, et al: Genetically engineered T cells expressing a HER2-specific chimeric receptor mediate antigen-specific tumor regression. Cancer Gene Ther 15:382-92, 2008

122. Overwijk WW, Theoret MR, Finkelstein SE, et al: Tumor regression and autoimmunity after reversal of a functionally tolerant state of self-reactive CD8+ T cells. J Exp Med 198:569-80, 2003

123. Klebanoff CA, Acquavella N, Yu Z, et al: Therapeutic cancer vaccines: are we there yet? Immunol Rev 239:27-44, 2011

124. Deol A, Lum LG: Role of donor lymphocyte infusions in relapsed hematological malignancies after stem cell transplantation revisited. Cancer Treat Rev 36:528-38, 2010

125. Tomblyn M, Lazarus HM: Donor lymphocyte infusions: the long and winding road: how should it be traveled? Bone Marrow Transplant 42:569-79, 2008

126. Klebanoff CA, Finkelstein SE, Surman DR, et al: IL-15 enhances the in vivo antitumor activity of tumor-reactive CD8+ T cells. Proc Natl Acad Sci U S A 101:1969-74, 2004

127. Rosenberg SA, Schwarz S, Spiess PJ: In vitro growth of murine T cells. II. Growth of in vitro sensitized cells cytotoxic for alloantigens. J Immunol 121:1951-5, 1978

128. Cheever MA, Greenberg PD, Fefer A: Specific adoptive therapy of established leukemia with syngeneic lymphocytes sequentially immunized in vivo and in vitro and nonspecifically expanded by culture with Interleukin 2. J Immunol 126:1318-22, 1981

129. Greenberg PD, Cheever MA, Fefer A: Eradication of disseminated murine leukemia by chemoimmunotherapy with cyclophosphamide and adoptively transferred immune syngeneic Lyt-1+2- lymphocytes. J Exp Med 154:952-63, 1981

130. Fisher B, Packard BS, Read EJ, et al: Tumor localization of adoptively transferred indium-111 labeled tumor infiltrating lymphocytes in patients with metastatic melanoma. J Clin Oncol 7:250-61, 1989

131. Rosenstein M, Eberlein T, Kemeny MM, et al: In vitro growth of murine T cells. VI. Accelerated skin graft rejection caused by adoptively transferred cells expanded in T cell growth factor. J Immunol 127:566-71, 1981

132. Rosenberg SA, Lotze MT, Muul LM, et al: A new approach to the therapy of cancer based on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2. Surgery 100:262-72, 1986

133. Muul LM, Spiess PJ, Director EP, et al: Identification of specific cytolytic immune responses against autologous tumor in humans bearing malignant melanoma. J Immunol 138:989-95, 1987

134. Rosenberg SA, Yang JC, Sherry RM, et al: Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res 17:4550-7, 2011

135. Besser MJ, Shapira-Frommer R, Itzhaki O, et al: Adoptive transfer of tumor-infiltrating lymphocytes in patients with metastatic melanoma: intent-to-treat analysis and efficacy after failure to prior immunotherapies. Clin Cancer Res 19:4792-800, 2013

136. Radvanyi LG, Bernatchez C, Zhang M, et al: Specific lymphocyte subsets predict response to adoptive cell therapy using expanded autologous tumor-infiltrating lymphocytes in metastatic melanoma patients. Clin Cancer Res 18:6758-70, 2012

137. Palmer DC, Chan CC, Gattinoni L, et al: Effective tumor treatment targeting a melanoma/melanocyte-associated antigen triggers severe ocular autoimmunity. Proc Natl Acad Sci U S A 105:8061-6, 2008

138. Ellebaek E, Iversen TZ, Junker N, et al: Adoptive cell therapy with autologous tumor infiltrating lymphocytes and low-dose Interleukin-2 in metastatic melanoma patients. J Transl Med 10:169, 2012

139. Andersen R, Donia M, Ellebaek E, et al: Long-Lasting Complete Responses in Patients with Metastatic Melanoma after Adoptive Cell Therapy with Tumor-Infiltrating Lymphocytes and an Attenuated IL2 Regimen. Clin Cancer Res 22:3734-45, 2016

140. Chandran SS, Somerville RP, Yang JC, et al: Treatment of metastatic uveal melanoma with adoptive transfer of tumour-infiltrating lymphocytes: a single-centre, two-stage, single-arm, phase 2 study. Lancet Oncol, 2017

141. Lee DW, Gardner R, Porter DL, et al: Current concepts in the diagnosis and management of cytokine release syndrome. Blood 124:188-95, 2014

142. Nishimura MI, Stroynowski I, Hood L, et al: H-2Kb antigen expression has no effect on natural killer susceptibility and tumorigenicity of a murine hepatoma. J Immunol 141:4403-9, 1988

143. Eisenhauer EA, Therasse P, Bogaerts J, et al: New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 45:228-47, 2009

144. Kochenderfer JN, Wilson WH, Janik JE, et al: Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. Blood 116:4099-102, 2010