Abbreviated Title: KK-LC-1 TCR T cells

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A Phase I Trial of T Cell Receptor Gene Therapy Targeting KK-LC-1 for Gastric, Breast, Cervical, Lung and other KK-LC-1 Positive Epithelial Cancers

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Drug Name:	KK-LC-1 TCR Transduced PBL	Fludarabine	Cyclophosphamide	IL-2 (Aldesleukin)
IND Number:	27348	27348	27348	27348
Sponsor:	CCR, NCI	CCR, NCI	CCR, NCI	CCR, NCI
Manufacturer:	CC DTM	Generic	Generic	Generic
Supplier:	CC DTM	CC Pharmacy	CC Pharmacy	CC Pharmacy

Investigational Device:

Name:	KK-LC-1 positivity assay
IDE #:	Nonsignificant Risk Device (NSR)
Sponsor:	CCR, NCI

Coordinating Center: NIH NCI Center for Cancer Research (CCR)
Participating Sites: See **Appendix H** for role of participating sites
Safety Monitoring Committee (SMC): NCI Intramural CCR SMC

PRÉCIS

Background:

- KK-LC-1 is a cancer germline (CG) antigen with expression restricted to germ cells (which lack MHC class I expression) in adults and epithelial cancers including lung, breast and gastric.
- This limited expression pattern makes it an ideal target for T Cell Receptor (TCR) gene therapy.
- TCR T cell therapy targeting CG antigens has been shown to induce objective responses without autoimmunity or off-target toxicity in participants with melanoma, synovial sarcoma and cervical cancer
- T cells genetically engineered with a TCR targeting KK-LC-1 display specific reactivity against HLA-A*01:01, KK-LC-1 target cells.
- KK-LC-1 TCR T cells can mediate tumor regression in pre-clinical mouse models of cancer

Objective:

- To determine the maximally tolerated dose of KK-LC-1 TCR T cells plus aldesleukin for the treatment of metastatic KK-LC-1 positive epithelial cancers.

Eligibility:

- Participants greater than or equal to 18 years old with metastatic or refractory/recurrent KK-LC-1 positive epithelial cancer.
- Prior first line systemic therapy is required unless the participant declines standard treatment.
- Participants must be HLA-A-*01:01-positive.

Design:

- This is a phase I clinical trial that will test the safety and efficacy of escalating doses of KK-LC-1 TCR T cells.
- Participants will receive a non-myeloablative lymphocyte-depleting preparative regimen of cyclophosphamide and fludarabine followed by a single infusion of KK-LC-1 TCR T cells and high-dose aldesleukin.
- Re-treatment will be allowed for a small number of subjects

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council for Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- To determine the maximally tolerated dose of KK-LC-1 TCR T cells plus aldesleukin for the treatment of metastatic KK-LC-1 positive epithelial cancers.

1.1.2 Exploratory Objectives

- To assess the safety and efficacy of KK-LC-1 TCR T cells plus aldesleukin for the treatment of KK-LC-1+ cancers.
- To conduct exploratory immunologic studies to understand and improve the administered treatment.

1.2 BACKGROUND AND RATIONALE

It is estimated that metastatic epithelial cancers are responsible for approximately 90% of cancer deaths [1-3]. The estimated 600,000 cancer deaths each year is driven largely by lung adeno- and squamous cell carcinoma and invasive breast cancer. These cancers account for approximately 30% of all cancer-related deaths in the United States **(Figure 1)** [4]. Furthermore, there are approximately 4,000 deaths per year due to cervical cancer and 10,000 from gastric cancer.

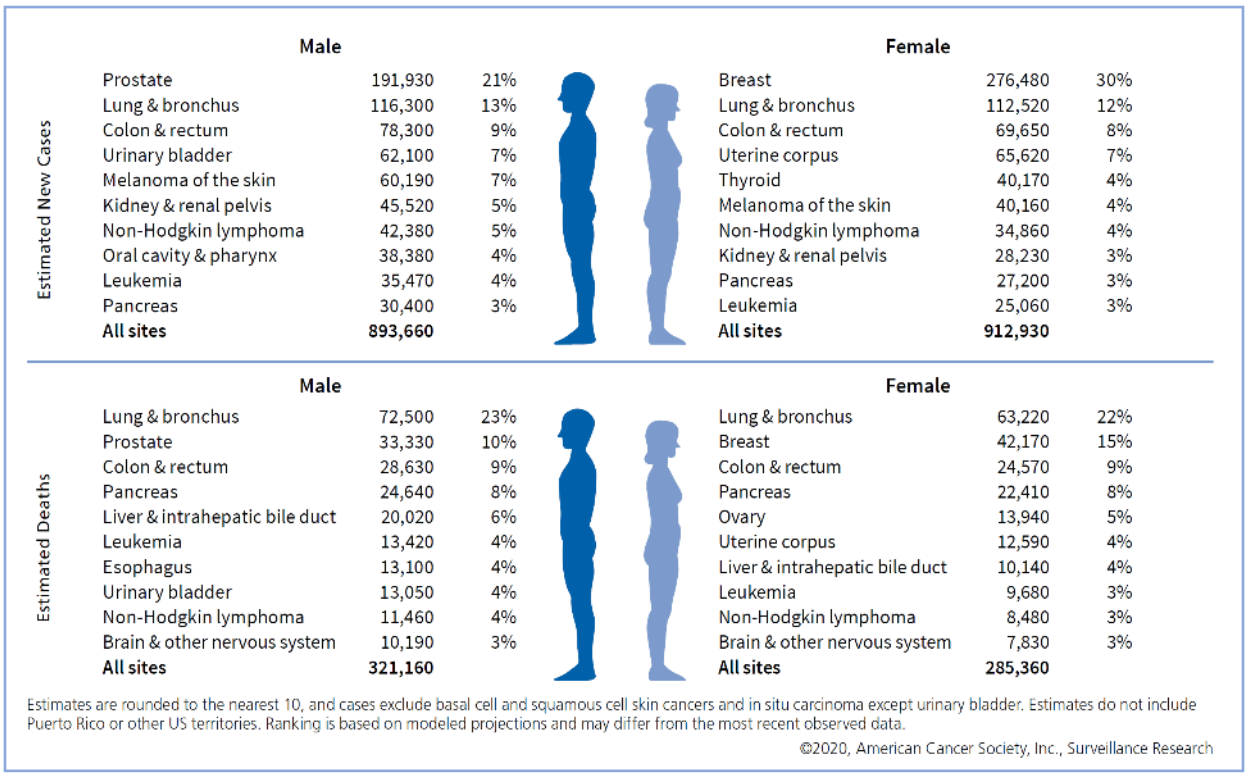


Figure 1**. Leading Sites of New Cancer Cases and Deaths – 2020 Estimates.**

The current treatment landscape for metastatic non-small cell lung cancer (NSCLC) has evolved over the past several years to include PD-1 based therapies. A recent, double-blind, phase 3 clinical trial demonstrated an improvement in the rate of overall survival at 12 months from 49% to 69% with the addition of pembrolizumab to standard platinum-containing chemotherapy as first-line treatment for metastatic, PD-L1 positive non-small cell lung cancer (NSCLC) [5]. Another recent phase 3 clinical trial demonstrated an improvement in median overall survival from 14.7 months to 19.2 months with the addition of atezolizumab and bevacizumab to first-line standard of care platinum-containing chemotherapy in participants with untreated metastatic, PD-L1 negative NSCLC [6]. Though progress has been made, the outlook for these participants remains poor with a 5-year relative survival rate of approximately 20% [4].

Metastatic, triple negative (ER/PR negative, HER2 negative) breast cancer portends a worse prognosis compared to metastatic, ER/PR positive breast cancer. Treatment with single agent chemotherapy is recommended for participants without germline BRCA 1/2 mutations or PD-L1-positive tumors. Clinical activity is modest with response rates anywhere from 10-40% [7-10]. In participants with PD-L1 positive disease, the addition of atezolizumab to single agent nab-paclitaxel resulted in a modest benefit in PFS of <2 months with a nonsignificant trend in improved median overall survival (17.6 vs 21.3 months) compared to single agent nab-paclitaxel [11]. A more substantial benefit in PFS and OS was seen in participants with tumors containing high populations of PD-L1-expressing immune effector cells [11]. Regardless, better therapies are still needed.

Like many other epithelial cancers, cervical cancer is incurable in the metastatic setting and difficult to palliate. Responses to chemotherapy are usually short-lived with a median PFS around 3 to 7 months [12, 13]. In a Gynecologic Oncology Group randomized trial comparing four cisplatin-based doublets as first line therapy for cervical cancer the response rates were 22 to 29 percent, median PFS was 4 to 6 months and median overall survival (OS) was 10 to 13 months [14]. The addition of bevacizumab to combination chemotherapy has been reported to increase overall survival by 3.7 months, but the vast majority of participants still die of their disease within 2 years [15]. Early evidence suggests that immune checkpoint therapy has only modest activity in this disease with a phase Ib trial (KEYSTONE 028) showing a 12.5% response rate (3/24 participants) and a phase II study showing a response rate of 13.3% (13/98 participants) to pembrolizumab [16].

Worldwide, gastric cancer remains a global health concern with nearly 1 million new cases diagnosed each year [17]. Approximately 75% of participants are diagnosed with metastatic disease, where median survival is around 12 months [18, 19]. The preferred treatment for metastatic gastric cancer includes a two-drug cytotoxic regimen consisting of a fluoropyrimidine (fluorouracil or capecitabine) and platinum (oxaliplatin or cisplatin) [20-25]. The survival benefit of chemotherapy over best supportive care is approximately 6.7 months [19]. Combination chemotherapy extends survival only slightly compared to single agent chemotherapy which is counterbalanced by an increase in toxicity [26]. In participants with HER2 overexpressing adenocarcinoma, the addition of trastuzumab to first-line chemotherapy improves overall survival by 2.7 months compared to chemotherapy alone (13.8 vs 11.1 months) [27]. Second-line treatments include single agent chemotherapy. Most data are from participants that were treatment-naïve or received one line of prior systemic therapy [28-30]. A randomized, placebo-controlled, double-blind, phase 3 trial comparing paclitaxel plus the VEGFR-2 antagonist ramucirumab to paclitaxel plus placebo demonstrated an improved overall survival of 2.2 months (9.6 vs 7.4 months) [31]. Pembrolizumab is recommended as second-line treatment for participants with MSI-H or dMMR tumors or as third-line therapy for adenocarcinomas with PD-L1 expression levels by CPS $\geq$1 where the response rate only reached 11.6% in the 259 participants treated [32-34]. More effective therapies are desperately needed.

1.2.1 Adoptive T cell Therapy

Adoptive T cell Therapy (ACT) with antigen receptor gene-engineered T cells that express chimeric antigen receptors (CARs) or T cell receptors (TCRs) is a promising cancer treatment. CARs have demonstrated robust clinical activity against multiple hematologic malignancies including acute lymphoblastic leukemia, non-Hodgkin lymphoma and multiple myeloma [35-37]. Translating this success to epithelial cancers has been a challenge [38, 39]. TCRs have shown clinical activity in melanoma and synovial cell sarcoma but success in epithelial cancers has been limited [39, 40]. A major challenge in targeting epithelial cancers is identification of a target antigen expressed on tumor cells that is not expressed by vital healthy tissue [41]. Initial efforts by our group focused on targeting human papillomavirus (HPV) associated epithelial cancers. HPV+ cancers are attractive candidates for T cell therapy because they express viral antigens that can be targeted by T cells [39]. The primary antigens for targeting with immunotherapy are the HPV E6 and E7 oncoproteins, which are viral proteins that are constitutively expressed by HPV+ cancers and not expressed by healthy human tissues. E6 and E7 contribute to malignant transformation and to survival of cancer cells, and this functional importance also makes them attractive therapeutic targets.

Our initial clinical trial employing TCR T cell therapy in HPV-associated cancers targeted the E6 antigen. To accomplish E6 targeting, autologous peripheral blood T cells were genetically engineered to express an HLA-A*02:01-restricted, HPV16 $E6_{29-38}$-specific T cell receptor (TCR) [42]. The trial was a phase I/II, dose-escalation study. Participants had metastatic HPV16+ cancer that in most participants was refractory to multiple systemic agents. Participants received a conditioning regimen of cyclophosphamide 60 mg/kg for two days and fludarabine 25 mg/m^2 for five days. Cell infusion was followed by high-dose aldesleukin. Two of 12 participants experienced partial tumor responses; one subsequently had residual disease resected by surgery, and she is without evidence of cancer years later. This trial validated the ability of T cell therapy targeting a single antigen to mediate regression of a metastatic epithelial cancer.

We are currently conducting a phase I/II clinical trial using a TCR targeting the E7 antigen. Similar to other T cell therapy trials, participants receive a conditioning regimen of cyclophosphamide 60 mg/kg or 30 mg/kg daily for two days and fludarabine 25 mg/m^2 daily for five days. Cell infusion is followed by high-dose aldesleukin. Thus far, objective tumor responses have been seen in 10 of 20 participants treated. Five of these responses were seen in participants previously treated with PD-1 based therapy. These responses included marked regression of bulky tumors in heavily pretreated participants **(Figure 2)**. This study provides further evidence of the potential of T cell therapy to mediate regression of widely metastatic, heavily pretreated epithelial cancers.

1.2.2 TCR T cell therapy targeting Cancer Germline Antigens

Cancer germline (CG) antigens are normally expressed predominantly by germ cells but can be expressed in cancers. However, because they do not express MHC class I molecules, germ cells are not recognized by TCRs. CG antigens can be subcategorized as testis restricted, testis and/or brain restricted, or testis selective based on their expression pattern. Testis-restricted and certain testis-selective CG antigens are rational targets for TCR T cell therapy [41].

Successful targeting of CG antigens including NY-ESO-1 and the MAGE family proteins has been demonstrated in previous clinical trials. In a phase I, cell dose escalation trial of an MHC class II-restricted TCR targeting MAGE-A3, a total of 17 participants were treated. A complete response was seen in one participant with metastatic cervical cancer that was ongoing for more than 29 months. At the highest dose level of cells, 3 of 9 participants experienced partial responses [43]. There were no off-target toxicities or targeting of healthy tissue. Another clinical trial tested an MHC class I-restricted TCR targeting the CG antigen NY-ESO-1. Responses were seen in 11 of 18 participants with synovial cell sarcoma and 11 of 20 participants with melanoma [40]. No autoimmune-mediated toxicities were seen.

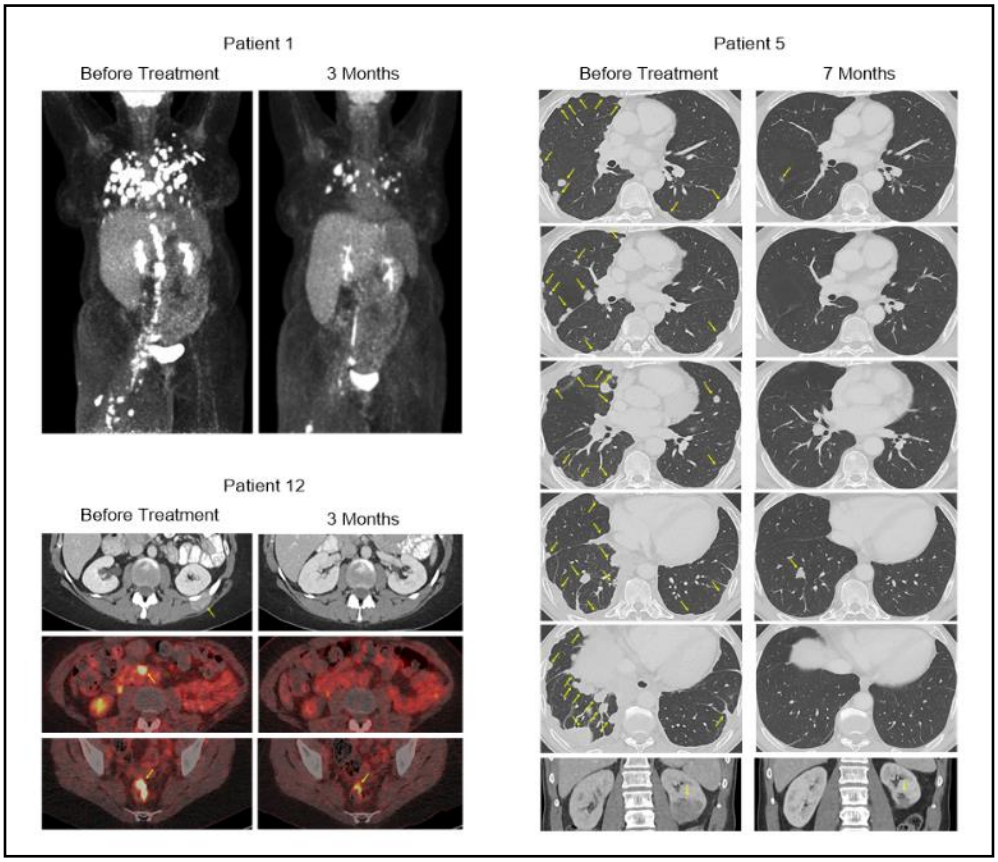


Figure 2. Clinical Activity of E7 TCR-T Cells.

Participant 1 had metastatic vulvar cancer with extensive pulmonary, retroperitoneal, pelvic and thigh lesions. Participant 5 had metastatic anal cancer with numerous pulmonary, pleural, and kidney lesions. Participant 12 had metastatic cervical cancer with chest wall, retroperitoneal, and rectal lesions.

1.2.3 The Kita-Kyushu Lung Cancer Antigen 1 (KK-LC-1)

KK-LC-1 (encoded by CT83) is a cancer germline antigen that is reported to have restricted expression in healthy tissue and frequent expression in certain epithelial cancers including lung, gastric and breast cancer [44-46]. It is the only member of its family, and therefore might be targeted without risk of intra-family cross-reactivity. Like most CG antigens, expression is regulated by epigenetic mechanisms that often result in coordinate gene expression. KK-LC-1 was identified as a potential immunotherapy antigen by characterization of the target of a lung adenocarcinoma-reactive T cell clone [44].

In a report published by our group, we examined the distribution of the CG antigen KK-LC-1 in normal tissue and epithelial cancers. CT83 expression was detected in the epididymis and testis, which lack HLA expression and thus, cannot be targeted by T cells, while absent from a broad range of normal tissue which included 51 non-neural and 24 neural tissues **(Figure 3A,B)** [47]. These data suggest that KK-LC-1 is a testis-restricted CG antigen. To investigate whether KK-LC-1 is expressed by epithelial cancers, bioinformatic analysis of The Cancer Genome Atlas (TCGA) Provisional data set accessed on the cBioPortal Cancer Genomics public database was performed. This analysis showed a wide range of cancers that expressed CT83 including testicular cancer, lung adenocarcinoma, pancreatic cancer, lung squamous cell carcinoma, cervical cancer, bladder cancer, head and neck cancer **(Figure 4A).** Furthermore, we

tested 57 cancer cell lines from 10 different types of cancer for CT83 expression by qRT-PCR. Lung, breast, cervical, ovarian, melanoma, prostate, and leukemia cancer cell lines were found to express CT83, albeit with varying levels and frequencies of expression **(Figure 4B).** We previously observed CT83 expression in an HPV+ metastatic cervical cancer. Examination of a bank of metastatic cervical cancer specimens revealed expression in 6/21 (29%) of cervical squamous cell carcinomas and 5/8 (63%) of cervical adenocarcinomas **(Figure 4C).** In other HPV+ cancers, expression was detected in 1/8 anal cancers, 0/5 head and neck cancers, and 0/2 vaginal cancers [47].

To assess the frequency of cells within a tumor that express CT83, we performed RNA in situ hybridization with RNAScope on a variety of epithelial cancers. The highest frequency of positive cells occurred in gastric cancers, of the 13 samples tested, 9 were positive for CT83 expression (median: 50%, range: 5 to 90%). Triple negative breast cancer and cervical cancer also had a high frequency of cells expressing CT83 **(**Figure 5**)**. Non-small cell lung cancer had a few samples with a high frequency of CT83 expressing cancer cells. These studies demonstrate that KK-LC-1 may be an attractive target for TCR-T cell therapy in participants with gastric, breast, cervical and potentially lung cancer [47].

1.2.4 Discovery and characterization of the KK-LC-1 TCR

We identified a KK-LC-1 TCR from the tumor-infiltrating lymphocytes (TILs) of a participant with cervical cancer who had a complete tumor response to TIL therapy (Figure 6) [48]. The KK-LC-1 clonotype containing the TCR was the most dominate clonotype in the infused TIL product with a frequency of 67%. This frequency exceeded that of the HPV-specific TCR clonotype by a factor of at least 10 across all time points measured (as far out as 10 months) **(Figure 7)** [48]. The relatively high frequency and persistence of the infused KK-LC-1 clonotype suggests that it might have contributed to cancer regression in this participant.

The KK-LC-1 TCR was found to target KK-LC-$1_{52\text{-}60}$, a naturally processed epitope, presented by the HLA-A*01:01 molecule [48]. Predicted binding of KK-LC-$1_{52\text{-}60}$ to other HLA molecules was weaker. The nucleotide sequence encoding the TCR was codon optimized for expression in human tissues and the TCR constant regions were swapped for their mouse counterparts, which in other receptors has improved TCR alpha/beta chain pairing. TCR expression was improved by reversing the order of the alpha and beta genes, and by making cysteine substitutions in the TCR constant regions and hydrophobic substitutions in the transmembrane region of the alpha chain constant region **(**Figure 8**)** [49, 50]. The TCR displayed recognition of as low as 10^{-3} µg/mL of the cognate peptide KK-LC-$1_{52\text{-}59}$ in the context of HLA-A*01:01+ target cells [51]. Peripheral blood T cells transduced to express the KK-LC-1 TCR display CD8-independent HLA-A*01:01/KK-LC-$1_{52\text{-}60}$ tetramer binding (**Figure 9**).

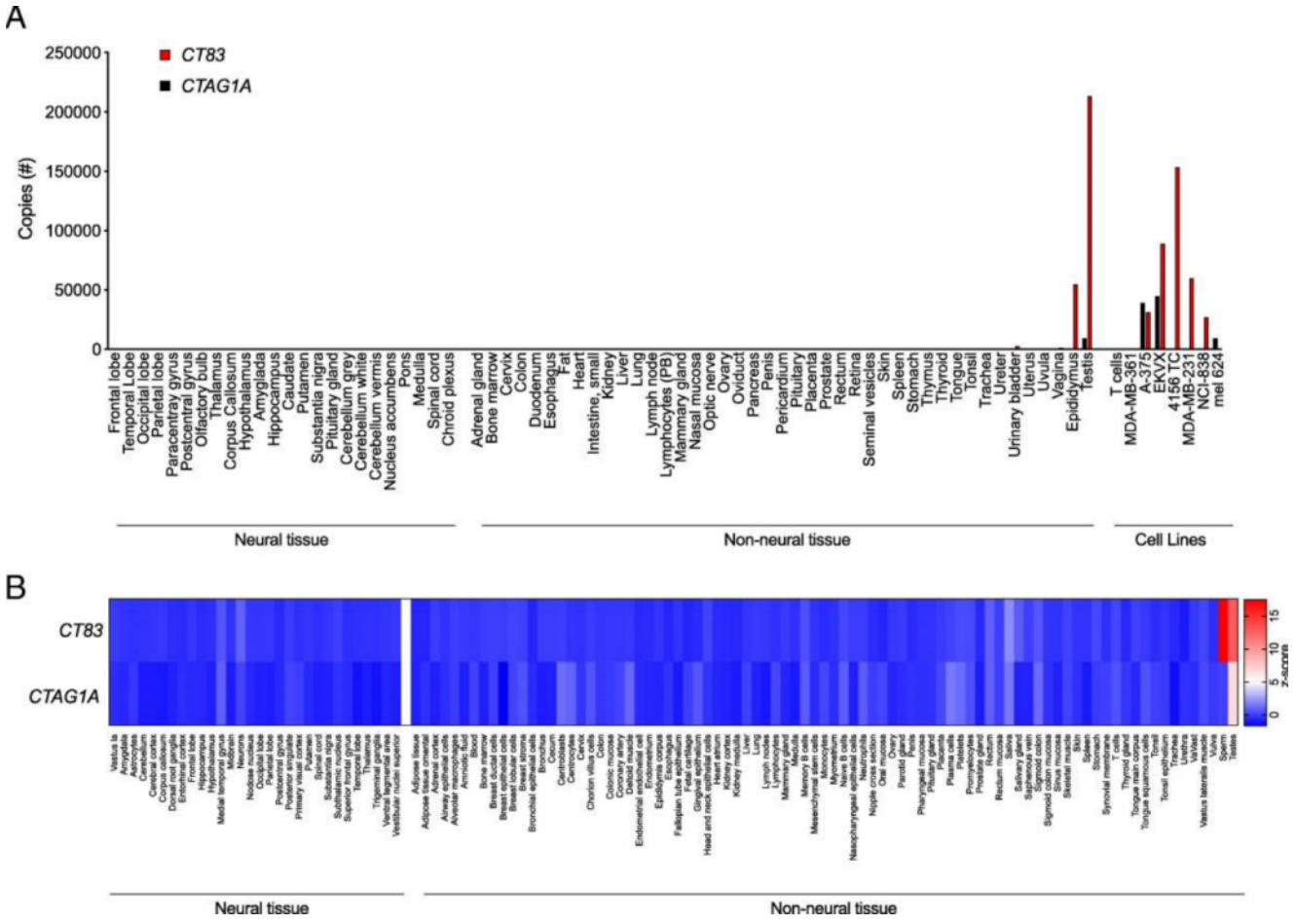


Figure 3. CT83 expression in healthy tissue. A. cDNA from a custom-made panel of 51 non-neural tissues and 24 neural tissues was assessed for CT83 and CTAG1A expression by qRT-PCR. cDNA from cancer cell lines and human T cells were included as controls. The y-axis displays total copy number. This experiment was performed twice with similar results. **B.** Healthy tissue expression of CT83 and CTAG1A is graphed as a heatmap based on data collected from the U133plus2 Affymetrix microarray available through BioGPS. The values shown are z-scores produced by the barcode function of the R package "frma." z-scores greater than 5 are suggestive of gene expression in the tissue.

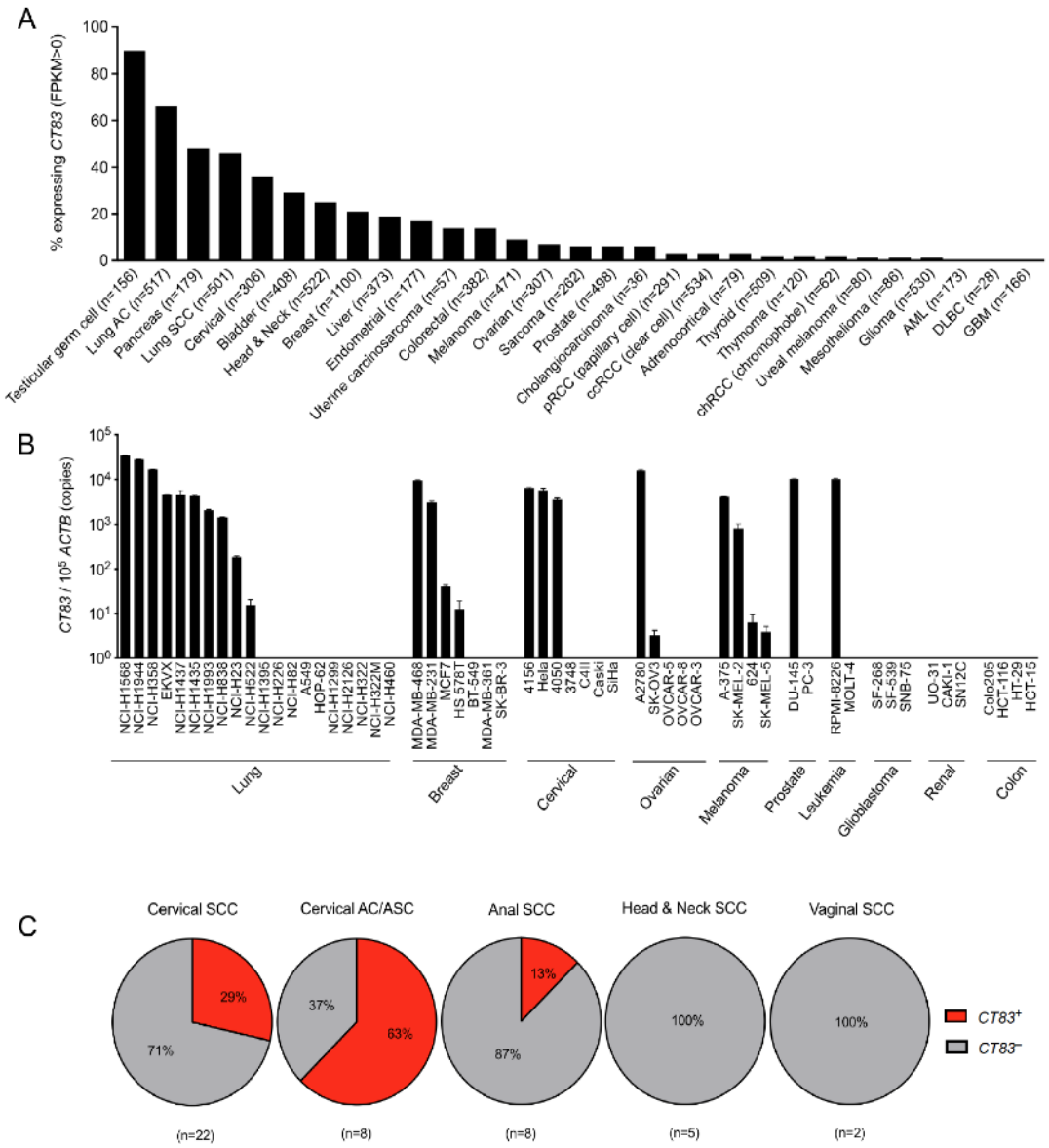


Figure 4. CT83 expression by cancer cell lines and tumors. A. The frequency (% of tumors that express the antigen) of CT83 expression by different cancer types was assessed. Expression data are derived from TCGA Provisional dataset accessed through cBioportal. Fragments Per Kilobase of transcript per Million mapped reads (FPKM) values > 0 were considered positive. The number of samples per cancer type is indicated in parentheses. **B.** A panel of cancer cell lines was assessed for CT83 expression by qRT-PCR. The y-axis displays CT83 copies per 10^5 copies of ACTB. This experiment was performed twice with similar results. **C.** The frequency of HPV+ metastatic cancers that express CT83 was assessed by qRT-PCR. The number of samples per cancer type is indicated in parentheses. Experiments were performed twice.

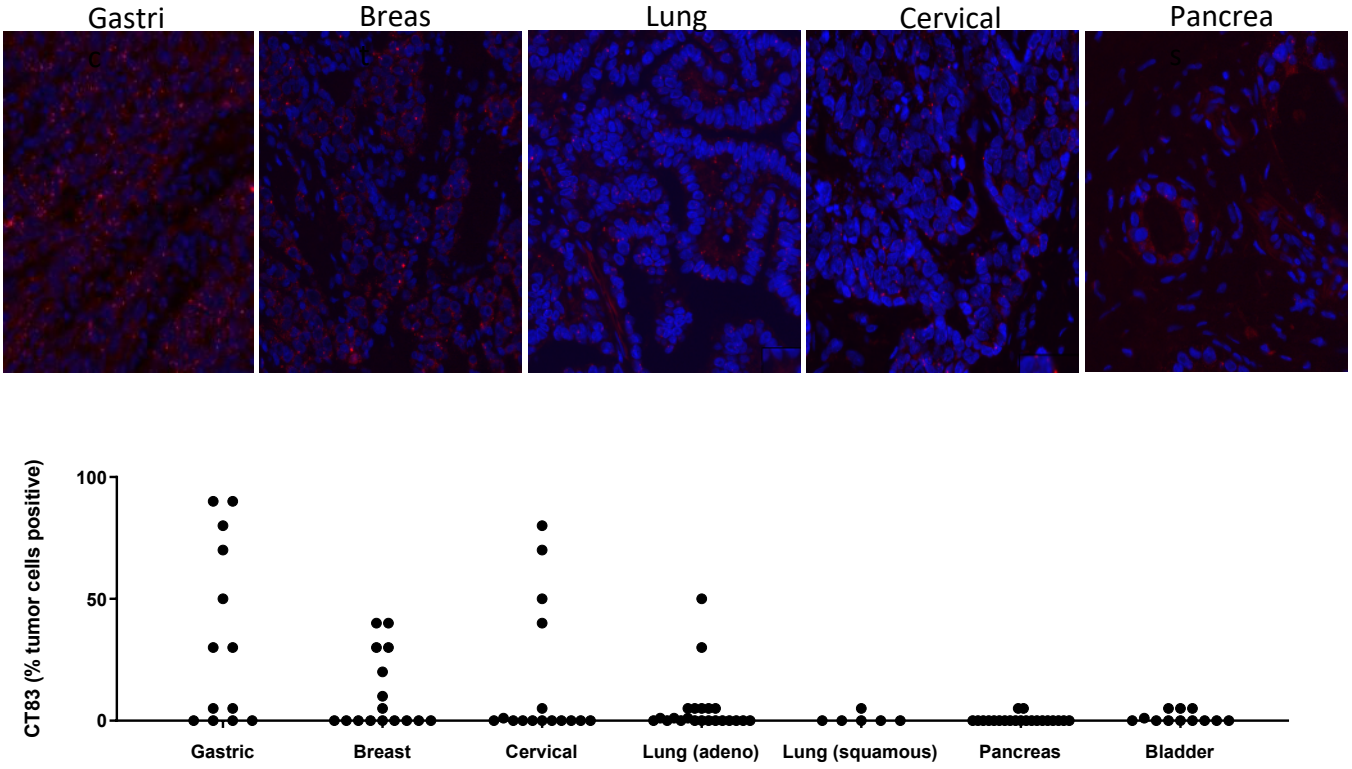


Figure 5. Intratumoral heterogeneity of CT83 expression. Top row depicts sample images of CT83+ tumors by RNAScope. The epithelial cancer is indicated above each image. The bottom row depicts the frequency of CT83+ tumor cells scored by an independent, blinded pathologist.

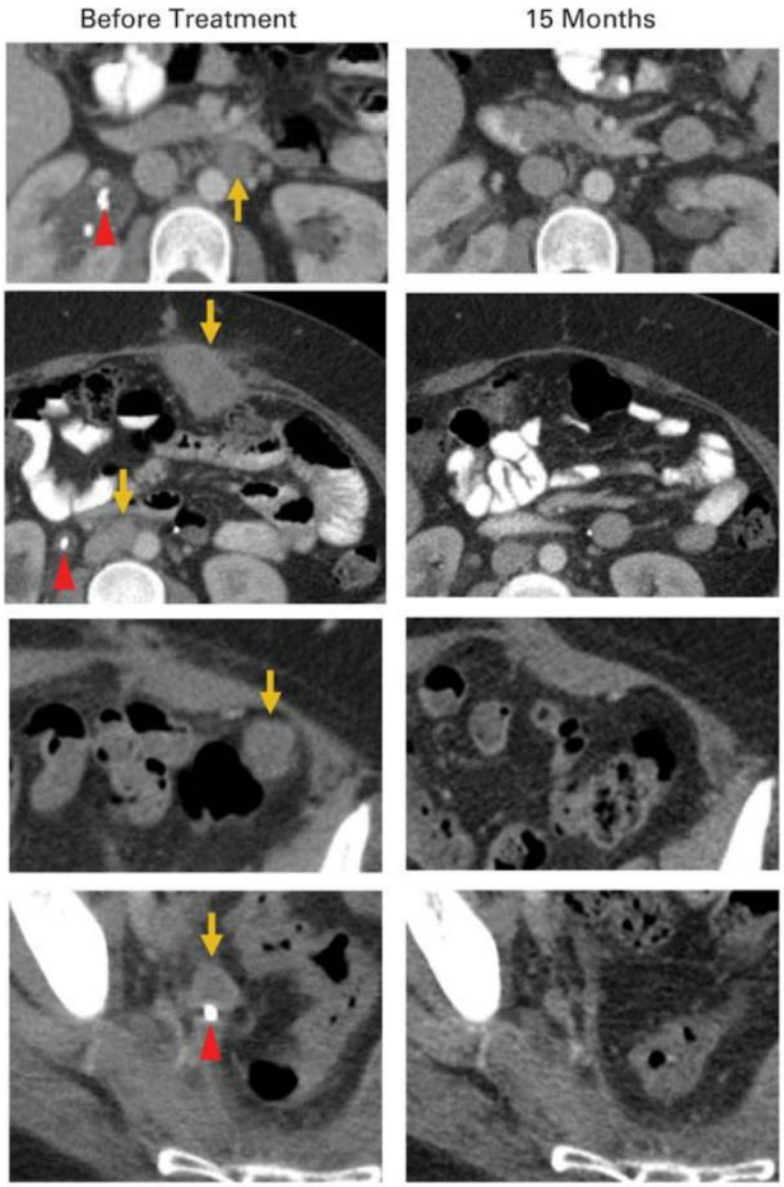


Figure 6. Participant was a 36-year-old female with metastatic cervical adenocarcinoma in the para-aortic lymph node, abdominal wall, aortocaval lymph node, left pericolic pelvic mass, and right ureteral nodule (gold arrows). She was treated with TIL therapy and had no evidence of disease 15 months after treatment. Red arrowhead indicates ureteral stent that was removed after right ureteral tumor regressed.

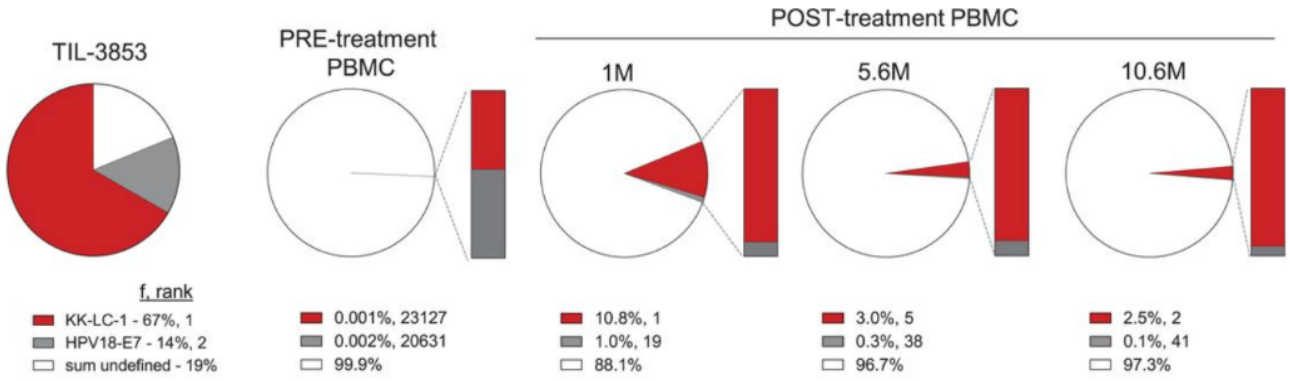


Figure 7. Frequency of KK-LC-1 clonotype in participant with complete response to TIL therapy. Frequency (f, %) and rank of individual tumor antigen–specific TCR clonotypes, as identified within TIL-3853 among PB mononuclear cells (PBMCs) before and after treatment (at the indicated months), were determined by TCRB deep sequencing. Posttreatment samples at 1 month during tumor regression and at 5.6 and 10.6 months during remission were analyzed. Pre indicates before treatment, Post indicates after treatment, and M indicates month(s).

We then tested to see if third-party human T cells that were transduced to express the KK-LC-1 TCR recognized tumor cell lines that express CT83 and HLA-A*01:01 *in vitro*. In overnight coculture assays, KK-LC-1 TCR-T cells from 2 donors displayed interferon (IFN)-γ release in response to cell lines that expressed the target antigen and the HLA restriction element, which indicated recognition of these lines **(Figure 10A)** [47]. These included the unmanipulated cell lines 4156 (cervical cancer), EKVX (lung cancer), and A375 (melanoma). All tested cell lines that expressed both the target antigen and the restriction element were recognized; conversely, all cell lines that did not express both the target antigen and the restriction element were not recognized.

To assess if systemically administered KK-LC-1 TCR-T cells could mediate tumor responses in vivo, we employed a murine xenograft model for the treatment of subcutaneous, established 4156 or A375 tumors. A single intravenous injection of KK-LC-1 TCR-T cells induced regression of 4156 tumors **(Figure 10B).** At the highest dose (10×10^6 cells) all mice demonstrated complete tumor regression. A375 tumors, which display heterogenous CT83 expression, eventually recurred, and recurrent tumors showed low CT83 expression, which may have contributed to their late relapse. Nonetheless, all mice with either 4156 or A375 tumors treated with at least $1x10^6$ KK-LC-1 TCR-T cells displayed tumor regression. These data indicate that KK-LC-1 TCR-T cells can target tumor cells in vitro and can mediate the regression of tumors in vivo [47].

We next evaluated KK-LC-1 TCR-T cells for cross-reactivity against potential epitopes of other human proteins. To determine which residues in the KK-LC-1_{52-60} epitope were critical for recognition by the KK-LC-1 TCR, we performed alanine and glycine scanning of the KK-LC-1_{52-60} peptide. Alanine substitutions at positions 3, 4, 5, 6, and 9 and glycine substitutions at positions 2, 3, 5, 6, 7, and 9 caused a greater than 75% decrease in IFN-γ release as compared to the wild type peptide. Based on these data, the residues at positions 3, 5, 6, and 7 were inferred to be the most essential non-anchor residues for TCR recognition (**Figure 11A,B**). The ScanProsite online tool was used to search for human proteins that shared these positions [52]. In addition, a Basic Local Alignment Search Tool (BLAST) search identified 6 more human peptides with high levels of sequence identity to KK-LC-1_{52-60}. KK-LC-1 TCR-T cells were tested for

recognition of the 10 candidate peptides in a coculture assay; recognition was not detected (**Figure 11C**). Thus, the KK-LC-1 TCR did not demonstrate detectable cross-reactivity against human peptides in vitro [47]. These characteristics demonstrate the potential efficacy and safety of KK-LC-1 TCR-T cells as a cancer therapeutic for participants with KK-LC-1+ epithelial cancers.

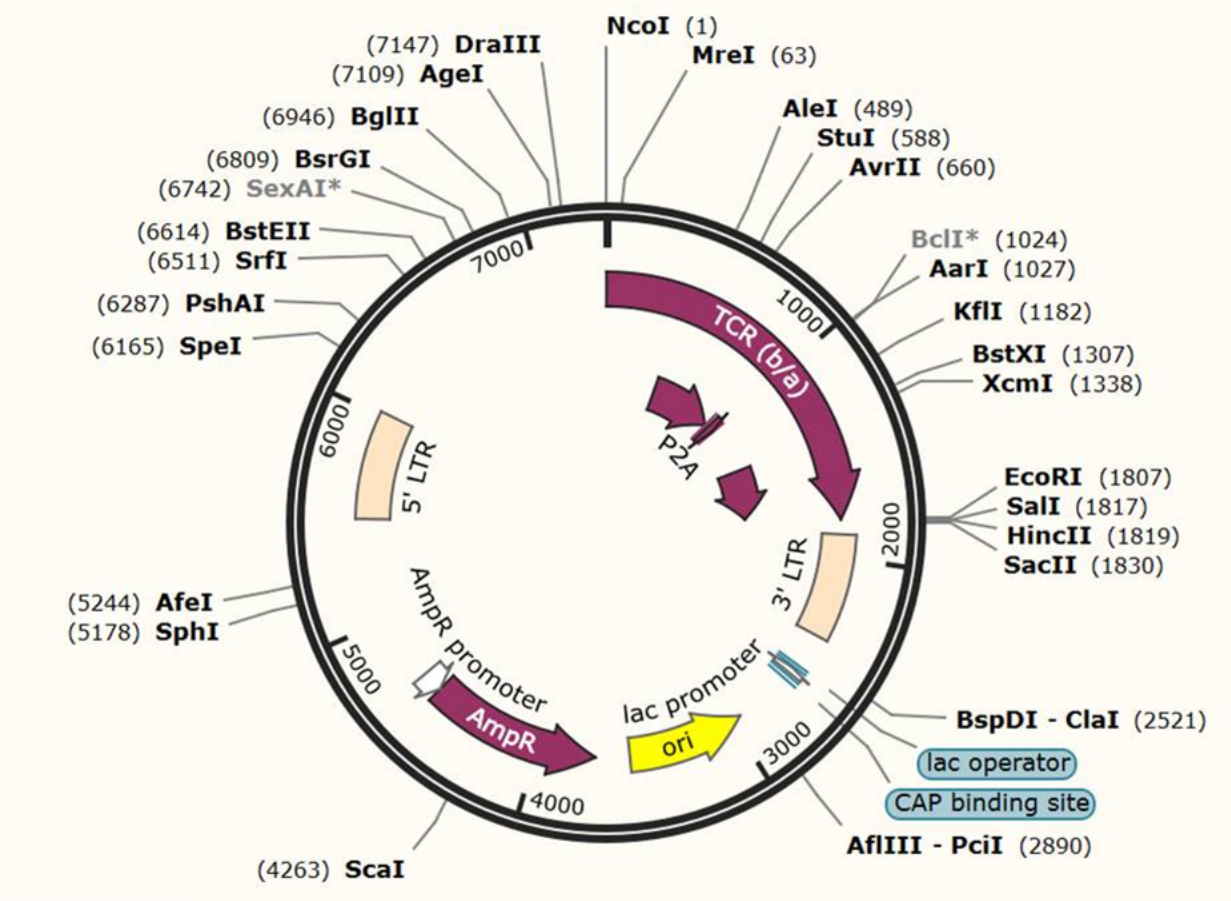


Figure 8. KK-LC-1 TCR vector map. A TCR targeting KK-LC-$1_{52\text{-}60}$ was isolated from a participant with a complete response to tumor-infiltrating lymphocyte therapy. The nucleotide sequence of the alpha and beta chains was determined, codon optimized for expression in human tissues, and the constant regions swapped for their mouse counterparts. A MSGV1 retroviral vector encoding this TCR was constructed. This retroviral vector consists of 7,313 base pairs and includes a 5'LTR from the murine stem cell virus (promoter), packaging signal including the splicing donor (SD) and splicing acceptor sites (SA). Alpha and beta chains of the KK-LC-1 TCR are linked by a furin 2A peptide.

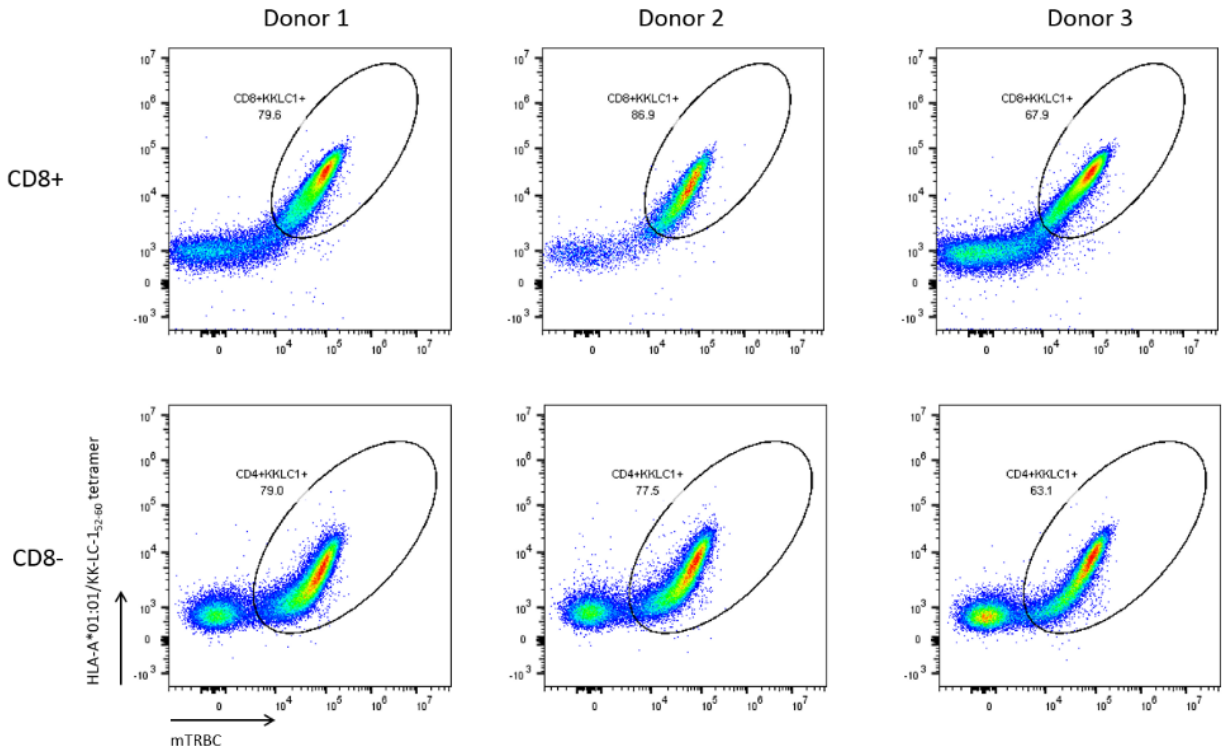


Figure 9: **Peripheral blood T cells transduced to express the KK-LC-1 TCR display CD8-independent HLA-A*01:01/KK-LC-1$_{52-60}$ tetramer binding.** T cells from PBMC were transduced to express the KK-LC-1 TCR. Dot plots shown are gated on PI- lymphocytes and either CD8+ or CD8- cells as indicated above the dot plots. The x-axis is mouse T cell receptor beta chain expression. The y-axis is tetramer binding.

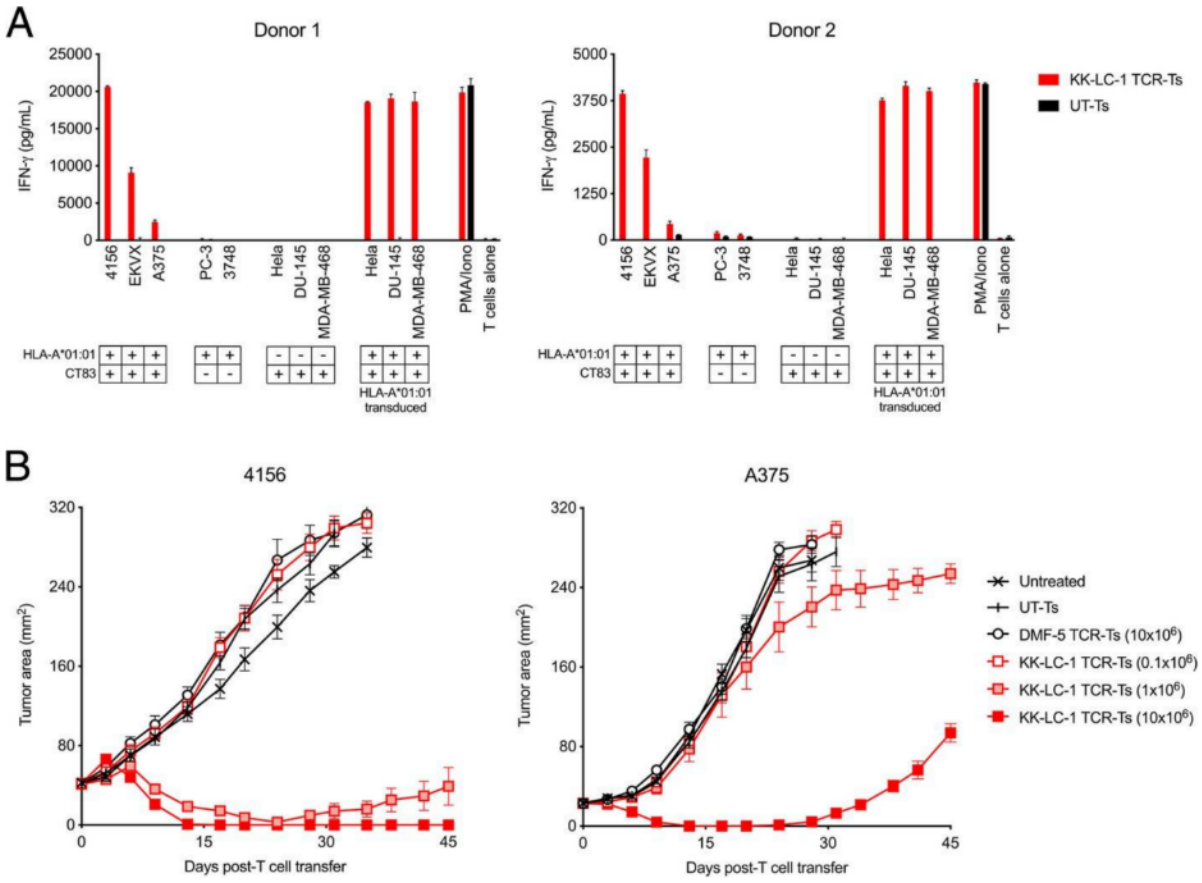


Figure 10. KK-LC-1 TCR-T cells display tumor recognition in vitro and mediate tumor regression in vivo. A. Human CD8+ T cells from each of 2 donors were transduced to express the KK-LC-1 TCR (KK-LC-1 TCR-T cells) or were not transduced (UT-Ts). Tumor recognition was tested in an overnight coculture assay with the target cell line indicated on the x-axis. The quantity of IFN-γ in the culture supernatants was determined by ELISA. Expression of CT83 and HLA-A*01:01 by each target cell line is indicated in the key below the x-axis. HLA-A*01:01 transduced cell lines were CT83+ and transduced with a γ-retrovirus to express HLA-A*01:01. "PMA/Iono" indicates T cells that were stimulated with PMA and ionomycin. "T cells alone" indicates T cells that were cultured without target cells or stimulation. **B.** KK-LC-1 TCR-T cells or control T cells indicated in the figure legend were administered intravenously to NSG mice bearing established 4156 or A375 subcutaneous tumors (as indicated above each graph). Serial tumor measurements were plotted at the timepoints indicated on the x-axis. Untreated mice did not receive any therapy. UT-Ts were not transduced. DMF-5 TCR-T cells target an irrelevant antigen (melanoma associated antigen-1) [53]. $N = 10$ mice per group. Error bars indicate the standard error of the mean. This experiment was performed twice with similar results

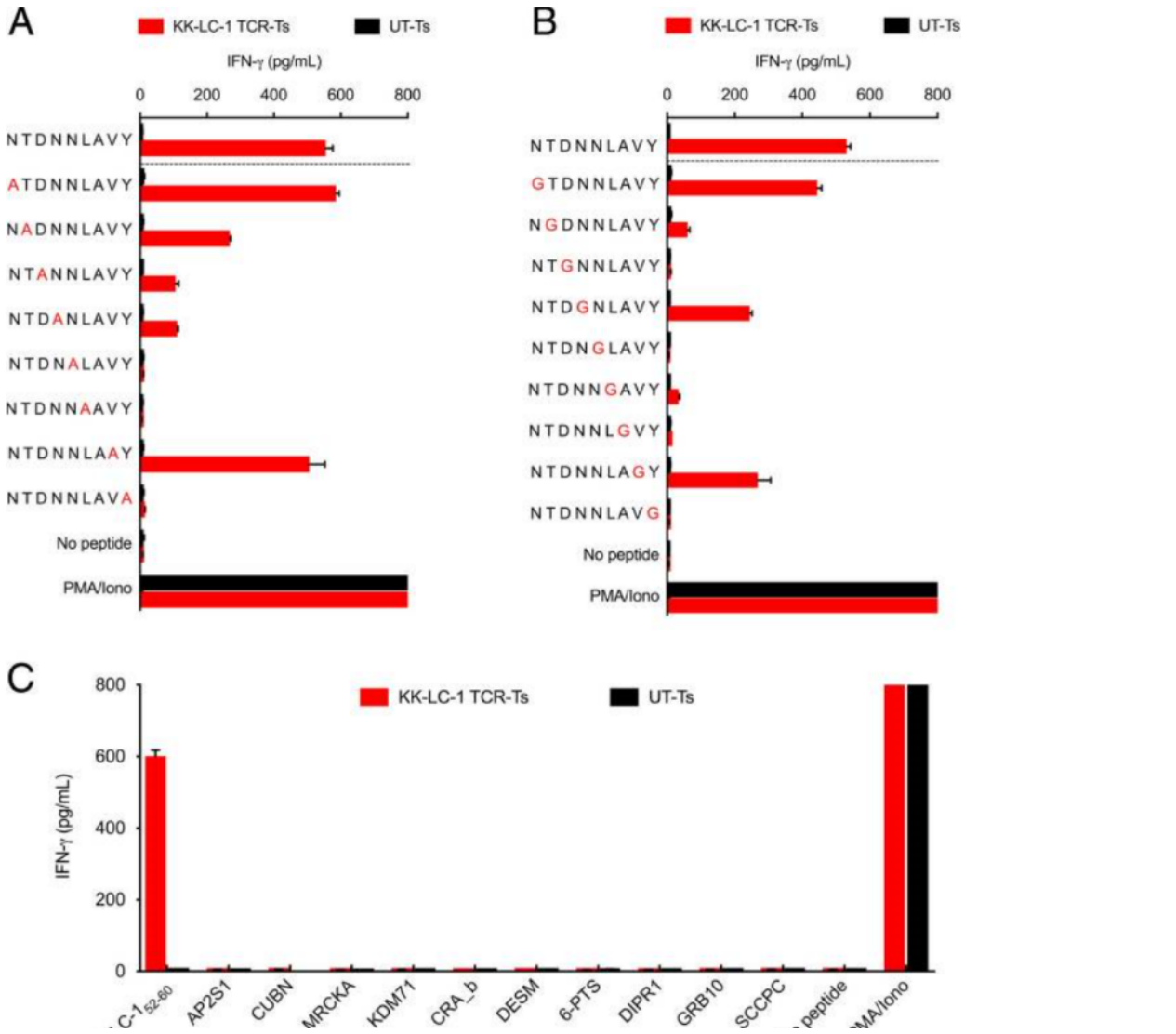


Figure 11. KK-LC-1 TCR-T cells did not demonstrate cross-reactivity with peptides derived from other human proteins. The IFN-γ production assays shown were performed by coculture of KK-LC-1 TCR-T cells with autologous EBV-LCLs loaded with 1 µg/mL of the peptide indicated. Coculture supernatants were harvested after overnight coincubation. IFN-γ concentration was determined by ELISA. Error bars represent the SD of 2 technical replicates. The "no peptide" conditions had target cells without peptide. "PMA/Iono" indicates T cells that were stimulated with PMA and ionomycin. "UT-Ts" were untransduced control T cells from the same donor as the KK-LC-1 TCR-T cells. A. To guide cross-reactivity testing, alanine scanning of KK-LC-1_{52-60} was performed. An alanine residue was substituted for the native residue at each position of KK-LC-1_{52-60}. B. To compliment alanine substitution and assess the influence of position 7 on target recognition, glycine scanning also was performed. Based on these data, the residues at positions 3, 5, 6, and 7 were inferred to be the most essential non-anchor residues for TCR recognition C. Peptides derived from human proteins that demonstrated identity at the contact residues inferred by the experiments in (a) and (b) or by a BLAST search for candidate peptides that shared at least 5/9 residues (55% identity) were tested for KK-LC-1 TCR-T recognition.

1.2.5 Safety Considerations

The safety of infusion of large numbers of retrovirally modified tumor reactive T cells has been demonstrated in prior clinical studies. Protocols at the NIH Clinical Center have administered over 1 X 10^{11} tumor infiltrating lymphocytes (TIL) with widely heterogeneous

reactivity including CD4, CD8, and NK cells. Experience at the NIH Clinical Center treating more than 200 participants with advanced cancers with genetically engineered T cells have not identified a risk of malignant transformation in this setting. The risk of insertional mutagenesis is a known possibility using retroviral vectors. It has been observed in the setting of CD34+ hematopoietic stem cells for the treatment of XSCID, WAS, and X-CGD. It has also been reported with lentiviral transduction in a participant who received CD19 CAR-T cells (it did not cause malignant transformation and may have enhanced the efficacy of the T cells) [54]. With retroviral vector-mediated gene transfer into mature T cells, there has been no evidence of malignancy due to genotoxicity since the first NCI sponsored gene transfer study in 1989. Although continued follow-up of all gene therapy participants will be required, data support the safety of retrovirally transduced mature T cells [55]. While the risk of insertional mutagenesis is low, the proposed protocol follows all current FDA guidelines regarding testing and follow up of participants receiving gene transduced cells [56]. Non-myeloablative chemotherapy conditioning and high-dose aldesleukin have well-characterized toxicity which is discussed in Section **12.4** of this study [57]. The chemotherapy used in this protocol has been administered to over 500 participants and all have reconstituted their hematopoietic system.

Toxicity from the KK-LC-1 TCR is unlikely because 1) the TCR came from a human and therefore has been selected in thymus to not have autoreactivity, so autoimmunity is unlikely, 2) the TCR specificity has not been altered by affinity enhancement so there is no chance that cross reactivity against normal human proteins has been introduced, 3) testing against human peptides with similarity to the target epitope and with human peptides identified by alanine and glycine scanning revealed no cross reactivity, 4) gene expression data suggests that expression in healthy tissue is restricted to immune privileged germ cells.

The starting cell dose of $1x10^8$ is the same starting dose used in other TCR T cell therapy trials at the NIH including an ongoing phase I/II trial of TCR T cell therapy targeting KRAS G12D (NCT03745326). Experience with solid tumors such as melanoma and synovial sarcoma indicate that T-cell numbers between $3x10^{10}$ and $>1x10^{11}$ may be required to achieve durable tumor regressions. Therefore, with a slow dose escalation, an undesirably high number of participants may be treated with suboptimal cell numbers while being exposed to the risks of conditioning chemotherapy. To avoid this, an escalation of 1 log will be employed starting at $1x10^8$ to a maximum cell dose of $6x10^{10}$. Furthermore, an in situ hybridization (ISH) assay will be used to measure the intratumoral heterogeneity of CT83 expression in a participant's tumor. This test may help us select participants with tumors that have homogenous expression of the target antigen and have a higher chance of receiving clinical benefit from this therapy while decreasing the chance of exposing a participant with tumors that have low or no expression of the target antigen to the risks of the conditioning chemotherapy. With Amendment A, an immunohistochemistry (IHC) assay will be used to select participants. Similar to the ISH assay, the IHC assay may help us select participants who may have a higher chance of receiving clinical benefit while decreasing the chance of exposing patients who may not receive benefit to the risks of conditioning chemotherapy. IHC is a reliable and common assay used in clinical medicine.

The same eligibility criteria used for the ISH assay (score of 25% or greater) will be used for the IHC assay.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Participants must have histologically or cytologically confirmed KK-LC-1 positive epithelial cancer (KK-LC-1 positivity assay performed at Rutgers Cancer Institute of New Jersey). KK-LC-1 expression will be determined by immunohistochemistry (IHC). KK-LC-1 score of 25% or greater will be considered positive.

2.1.1.2 Participants must be HLA-A*01 by low resolution typing, and HLA-A*01:01 by one of the high resolution type results

2.1.1.3 Measurable (per criteria in section **6.3**) metastatic or refractory/recurrent KK-LC-1+ epithelial cancer (determined by IHC).

2.1.1.4 All participants must have received prior first line standard therapy or be ineligible to receive available therapies with known survival benefit.

2.1.1.5 Participants with three or fewer brain metastases that have been treated with surgery or stereotactic radiosurgery are eligible. Lesions that have been treated with stereotactic radiosurgery must be clinically stable for one month before protocol treatment. Participants with surgically resected brain metastases are eligible.

2.1.1.6 Age ≥18 years of age. Because no dosing or adverse event data are currently available on the use of KK-LC-1 TCR in participants <18 years of age, children are excluded from this study.

2.1.1.7 ECOG performance status ≤1 (**Appendix D**).

2.1.1.8 Participants must have adequate organ and marrow function as defined below:

– leukocytes	≥3,000/mcL
– absolute neutrophil count	≥1,500/mcL
– platelets	≥100,000/mcL
– hemoglobin	≥9.0 g/dL
– total bilirubin	within normal institutional limits
– AST(SGOT)/ALT(SGPT)	≤2.5 X institutional upper limit of normal
– creatinine	within normal institutional limits
	OR
– creatinine clearance	≥60 mL/min/1.73 m^2 for participants with

creatinine levels above institutional normal.

2.1.1.9 Serology:

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

2.1.1.10 The effects of the study agent on the developing human fetus are unknown. For this reason and because other therapeutic agents used in this trial are known to be teratogenic, individuals of child-bearing potential and their partners must agree to use adequate contraception (barrier method of birth control; abstinence) prior to study entry and up to twelve (12) months after treatment. Individuals of childbearing potential must have a negative pregnancy test. Individuals of childbearing potential are defined as all individuals except individuals who are postmenopausal or who have had a hysterectomy. Postmenopausal will be defined as individuals over the age of 55 who have not had a menstrual period in at least one year or a participant that has received a treatment (i.e., chemotherapy or pelvic radiation) that has resulted in them becoming postmenopausal. Should an individual become pregnant or suspect pregnancy while individuals or partner is participating in this study, individuals should inform treating physician immediately.

2.1.1.11 Because there is a potential risk for adverse events in nursing infants secondary to treatment of the mother with KK-LC-1 TCR transduced PBL, breastfeeding should be discontinued if the mother is treated with KK-LC-1 TCR transduced PBL. These potential risks may also apply to other agents used in this study.

2.1.1.12 More than four weeks must have elapsed since any prior systemic therapy at the time the participant receives the KK-LC-1 TCR T cells.

2.1.1.13 Participants may have undergone minor surgical procedures within the past three weeks, as long as all toxicities have recovered to Grade 1 or less or as specified in the eligibility criteria in Section **2.1**.

2.1.1.14 Ability of subject to understand and the willingness to sign a written informed consent document.

2.1.2 Exclusion Criteria

2.1.2.1 Participants who are receiving any other investigational agents.

2.1.2.2 History of severe immediate hypersensitivity reaction to cyclophosphamide, fludarabine or aldesleukin.

2.1.2.3 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with

study requirements. Participants with abnormal pulmonary function tests but stable obstructive or restrictive pulmonary disease may be eligible.

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

2.1.2.5 Concurrent opportunistic infections (The experimental treatment being evaluated in this protocol depends on an intact immune system. Participants who have decreased immune competence may be less responsive to the experimental treatment and more susceptible to its toxicities).

2.1.2.6 Participants with autoimmune diseases such as Crohn's disease, ulcerative colitis, rheumatoid arthritis, autoimmune hepatitis or pancreatitis, and systemic lupus rythematosus. Hypothyroidism, vitiligo and other minor autoimmune disorders are not exclusionary.

2.1.2.7 Participants on active systemic immunosuppressive therapy that cannot be safely withheld.

2.1.2.8 Participants with a history of coronary revascularization or ischemic symptoms unless participant has a normal cardiac stress test.

2.1.2.9 Any condition which would, in the opinion of the Principal Investigator, indicate that the subject is a poor candidate for the clinical trial or would jeopardize the subject or the integrity of the data obtained.

2.1.2.10 Documented LVEF $\leq$ 45%

2.2 RECRUITMENT STRATEGIES

Participants for this protocol will be recruited via standard CCR mechanisms including posting to NIH websites (i.e., clinicaltrials.gov) and social media platforms, physician and self-referrals as well as various advertising venues. All advertisements, letters and other recruitment efforts will be submitted to the IRB for approval prior to their implementation.

2.3 SCREENING EVALUATION

2.3.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes

2.3.2 Screening activities performed after a consent for screening has been signed

The following activities will be performed only after the subject has signed the consent for this study for screening. Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a participant has signed the consent.

2.3.2.1 Within 24 hours prior to screening activities:

- Negative pregnancy test

2.3.2.2 Any time prior to starting the chemotherapy regimen:

a. HLA typing**

b. KK-LC-1 testing of tumor. Archival tissue may be used or a new biopsy may be obtained***

c. Venous assessment (as per apheresis clinic policy)

2.3.2.3 Within 4 weeks prior to starting the chemotherapy regimen:

a. Complete history and physical examination, including weight, and vital signs noting in detail the exact size and location of any lesions that exist. (Note: Participant history may be obtained within 8 weeks.)

b. EKG

c. Pulmonary Function Testing for participants with a prolonged history of cigarette smoking (20 pack/year of smoking within the past 2 years) or symptoms of respiratory dysfunction. (Note: If performed within 1 year of screening, this does not need to be repeated unless clinically indicated. Test results from outside the CCR may be accepted per investigator discretion).

d. If available, previous CT of the chest, abdomen and pelvis, and brain MRI or PET to evaluate the status of disease. Additional scans and x-rays may be performed if required to determine participant eligibility or if clinically indicated based on participants' signs and symptoms. (Note: Test results from outside the CCR may be accepted per investigator discretion if performed within 3 months of screening).

e. Cardiac evaluation for participants who are greater than or equal to age 50, or who have a history of ischemic heart disease, chest pain, or clinically significant atrial and/or ventricular arrhythmias including but not limited to: atrial fibrillation, ventricular tachycardia, heart block. Participants with a LVEF of less than or equal to 45% will not be eligible. Participants under the age of 50 who present with cardiac risk factors may undergo cardiac evaluation (e.g., diabetes, hypertension, obesity.) (Note: Test results from outside the CCR may be accepted per investigator discretion if performed within 6 months of screening).

f. HIV antibody titer and HbsAG determination, anti-HCV. (Note: may be performed within 3 months of chemotherapy start date but must be within one month before initial apheresis for generation of a cell product).

g. Anti CMV antibody titer, HSV serology, and EBV panel. (Note: participants who are known to be positive for any of the above do not need to be retested; may be performed within 3 months of chemotherapy start date).

2.3.2.4 Within 14 days prior to starting the chemotherapy regimen:

a. Blood tests:

- Complete Blood count with differential

- Chemistries: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (Bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, and Total Protein.
- Thyroid Panel

b. Urinalysis and culture if indicated

2.3.2.5 Within 7 days prior to starting the chemotherapy regimen:

a. Beta-HCG pregnancy test (serum or urine) on all individuals of child-bearing potential

b. ECOG performance status of 0 or 1

2.4 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g. when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

2.4.1 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

2.4.2 Treatment Assignment Procedures

Cohorts

Number	Name	Description
1	*Phase I: Dose Escalation Cohort*	*Evaluable participants with KK-LC-1 positive epithelial cancer to determine MTD of KK-LC-1 TCR T cells plus aldesleukin*

Arms

Number	Name	Description
1	*Treatment at dose levels 1 through 6*	*Non-myeloablative, lymphocyte depleting preparative regimen, followed by KK-LC-1 TCR T cells plus aldesleukin at escalating doses*

Arm Assignment

Participants in Cohort 1 will be directly assigned to Arm 1.

2.5 BASELINE EVALUATION

The following are baseline evaluations. Some of the screening evaluations may be used as baseline.

2.5.1 Within 4 weeks prior to starting the chemotherapy regimen:

a. Participants with lesions that can be biopsied under sedation and/or local anesthesia (skin lesions, visceral lesions approachable by CT, USG or MRI guided core biopsy) and who are willing to have biopsies performed, will have a baseline pre-treatment biopsy followed by two additional biopsies, one following treatment (6 weeks post treatment preferred) and the second at the time of progression. Refer to Section **5.1.3** for guidelines for handling specimens.

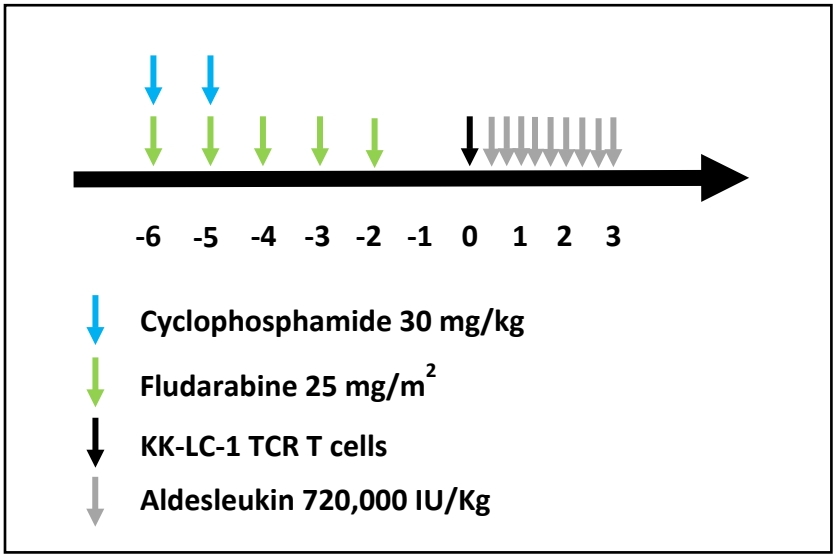
b. CT of the chest, abdomen and pelvis, PET scan and brain MRI may be performed to evaluate the status of disease.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a single-center, phase I clinical trial that will determine a safe dose of KK-LC-1 TCR T cells. Participants will receive a non-myeloablative lymphocyte-depleting preparative regimen of cyclophosphamide and fludarabine followed by a single infusion of KK-LC-1 TCR T cells and high-dose aldesleukin.

A small number of subjects may be eligible for re-treatment, and would be required to meet all eligibility criteria at the time of re-enrollment. Participants will be assigned a new sequential study number for the re-enrollment study period. Any cryopreserved cells produced from a participant who was removed from the study can be used to treat that participant after re-enrollment. We do not anticipate changes in the risk profile for the initial versus re-enrollment.



*Details regarding the number of participants in each dose level group are provided in section **3.1.5**.

3.1.1 Leukapheresis

The participant will undergo a 10-15 liter leukapheresis (generally, 12 liters will be processed to target a yield of 6-10 $x10^9$ lymphocytes) in the Department of Transfusion Medicine (DTM) Dowling Apheresis Clinic according to DTM standard operating procedures. This procedure may occur on this protocol, or protocol 16C0061, if the participant chooses to co-enroll on that protocol. 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. Leukapheresis material that is not required for clinical use may be retained and cryopreserved in 10 vials at $100x10^6$ cells per vial with remaining cells stored at $300x10^6$ cells per vial for research and banked on protocol 16C0061 (Tissue Procurement Protocol).

3.1.2 KK-LC-1 TCR T cell preparation

After cells are obtained by apheresis (either on this protocol or protocol 16C0061 if the participant has co-enrolled on that protocol), further cell processing to generate KK-LC-1 TCR T cells will occur in the CCE according to standard operating procedures and the KK-LC-1 TCR investigational new drug application. If apheresis has been performed on protocol 16C0061 and the participant consents and is eligible for treatment on this study, cells will be transferred to this study and all cell preparation will occur as part of this protocol. Any unused cells from this protocol can be transferred to 16C0061 and banked for research if a participant is co-enrolled. KK-LC-1 TCR T cells can be produced in approximately 11 to 15 days. Cell products may be cryopreserved during production to accommodate participant treatment schedules. Either freshly-collected cells or cryopreserved cells can be used to initiate the cell-preparation process. Peripheral blood mononuclear cells (PBMC) will be isolated. Sufficient cells for three complete cell productions (10 vials at 3-4.5 x 10^8 cells/vial) will be retained in the CCE; the remaining cells may first be frozen in 10 vials at 100 x 10^6 cells per vial with excess frozen at 300 x 10^6 cells/vial. Excess PBMC may be transferred from DTM to the Blood Processing Core (BPC) where they may be frozen and stored. The contact for the Blood Processing Core (BPC): pager #11964 and telephone # 240-760-6180.

Before infusion, the percentage of T cells expressing the KK-LC-1 TCR will be determined by flow cytometry. In addition to flow cytometry, further testing of the cells will take place prior to infusion to evaluate for microbial contamination, replication-competent retroviruses, and viability. Details of this testing can be found in the appropriate DTM SOPs. When a participant is no longer eligible for retreatment on this protocol due to meeting any of the off-study criteria listed in Section **3.9**, any remaining cryopreserved pretreatment PBMC collected on this protocol may be transferred from the Center for Cellular Engineering to the Principal Investigator of this protocol for storage in the Blood Processing Core (BPC) and possible use in research and banked according to protocol 16C0061.

3.1.3 Treatment Phase

PBMC will be obtained by leukapheresis (approximately $2x10^9$ to $1x10^{10}$ cells are obtained). PBMC will be cultured in the presence of anti-CD3 (OKT3) and aldesleukin in order to stimulate T-cell growth. Transduction is initiated by exposure of approximately $10x10^6$ to $500x10^6$ cells to supernatant containing the KK-LC-1 TCR retroviral vector. These transduced cells will be expanded and tested for their anti-tumor activity. Successful TCR gene transfer will be determined by FACS analysis for the TCR protein. Successful TCR gene transfer for each transduced peripheral blood lymphocyte (PBL) population will be defined as greater than 15%

TCR positive cells. A central line catheter may be used for the intravenous infusion of KK-LC-1 TCR T cells.

Prior to receiving the engineered PBL cells, participants will receive a non-myeloablative but lymphocyte depleting preparative regimen consisting of cyclophosphamide and fludarabine, on days -6 to -2 before the intravenous infusion of *in vitro* tumor reactive TCR gene-transduced PBL plus IV high dose aldesleukin, as indicated in Section **3.2**. Participants will receive one course of treatment. The start date of the course will be the start date of the chemotherapy; the end date will be the day of the first post-treatment evaluation and the first safety and response assessment at the first follow-up visit.

3.1.4 Dose Limiting Toxicity

The dose-limiting toxicity (DLT) evaluation period for determination of the maximum tolerated cell dose (MTD) will begin at the time of cell infusion and 30 days after cell infusion. A DLT is defined as follows: All grade 3 and greater toxicities related to cell infusion, with the exception of:

- Cytokine Release Syndrome (CRS) that resolves ≤ grade 2 within 14 days of the last dose of aldesleukin
- Autoimmune toxicity that resolves ≤ grade 2 within 14 days from starting symptoms treatment (e.g. steroids)
- Cardiac, gastrointestinal, dermatological, hepatic, pulmonary, renal, hematologic, neurologic toxicity, or toxicity in **Appendix B** attributable to aldesleukin that resolves to ≤ grade 2 within 14 days of the last dose of aldesleukin
- Transient grade 3 hypoxia associated with cell infusion that corrects to ≤ grade 2 with supplemental oxygen and/or that resolves to ≤ grade 2 within 24 hours
- Hemorrhage due to underlying cancer or prior radiotherapy
- Infection that is controlled within 7 days of onset
- Hematological toxicities because they are expected from the conditioning regimen and their duration is unpredictable
- Grade 3 metabolic laboratory abnormalities without significant clinical sequela that resolve to grade 2 within 7 days
- Grade 3 fever that resolves ≤ grade 2 within 14 days of cell infusion
- If a DLT is clearly due to progressive disease the subject will be replaced and the DLT will not be included

3.1.5 Dose Escalation

Participants will be entered in sequential dose levels and receive escalating doses of cells beginning at 1 x 10^8. The protocol will enroll one participant in each dose level unless a participant experiences a DLT. A total of 12 participants will be enrolled at the MTD or highest safe dose level. The total number of transduced KK-LC-1 TCR T cells infused at each dose level will be:

Dose Escalation Schedule	
Dose Level	Dose of IND Agent
Level 1	1 x 10^8 transduced KK-LC-1 TCR T cells
Level 2	5 x 10^8 transduced KK-LC-1 TCR T cells
Level 3	1 x 10^9 transduced KK-LC-1 TCR T cells
Level 4	5 x 10^9 transduced KK-LC-1 TCR T cells
Level 5	1 x 10^{10} transduced KK-LC-1 TCR T cells
Level 6	6 x 10^{10} transduced KK-LC-1 TCR T cells

The cell dose administered will be in a range of +/- 30% of the target dose above. The number of transduced cells will be quantified by multiplying the frequency of cells expressing the mouse TCR constant region (determined by flow cytometry) by the number of cells produced. If fewer than the target number of cells are generated the participant will still be treated but toxicity data from the participant will not be used in determining the MTD.

In each dose level, if a participant experiences a DLT, a total of 6 participants will be treated at that dose level to confirm that no greater than 1 of 6 participants have a DLT prior to proceeding to the next higher dose level. If a level with 2 or more DLTs in 6 participants has been identified, participants will be accrued at the next-lowest dose, for a total of 6 participants. If 2 DLTs occur in the first dose level, the study may be amended to treat participants at lower doses.

The MTD is the highest dose at which ≤ 1 of 6 participants experienced a DLT or the highest dose level studied if 2 DLTs are not observed at any of the dose levels. Six more participants will be enrolled at the MTD or highest safe dose explored in order to obtain more toxicity information and to provide additional participants for the exploratory efficacy evaluations. Thus, a total of 12 participants will be treated at the MTD or highest dose level explored.

There will be a 30-day delay between each dose level in order to determine if the participant experiences a DLT. There will be a 14-day delay in treatment between participants within each dose level. Therefore, after a participant in a dose level starts chemotherapy, the next participant in the same dose level will not start chemotherapy until at least 14 days after the start of chemotherapy to allow more time for the analysis of adverse events.

3.1.6 Safety Protocol Stopping Rules

The study will be halted (immediately stop accrual and treatment) if any of the following safety conditions are met and we will promptly investigate and submit an amendment to the IRB and FDA if necessary:

1. If one or more deaths (other than death related to progressive disease) occurs within 30 days of treatment regimen.
2. If two or more participants develop a Grade 3 or greater toxicity related to the cell product that does not resolve to grade 2 within 10 days, with the exception of:

a. Grade 3 metabolic laboratory abnormalities without significant clinical sequela that resolve to grade 2 or less within 14 days

b. Grade 3 fever that resolves < grade 2 within 14 days of cell infusion

3. If 2 DLTs occur on dose level 1.

3.2 DRUG ADMINISTRATION

3.2.1 Preparative Regimen

The following will comprise a course of therapy:

Drug	Dose	Route and Administration	Treatment Days
Cyclophosphamide	30 mg/kg	IV infusion over 1 hour (± 10 min)	Once daily x 2 doses Days -6 and -5
Fludarabine	25 mg/m^2	IV infusion over 30 minutes (± 10 min) To be administered following completion of cyclophosphamide.	Once daily x 5 doses Days -6, -5, -4, -3, -2

Dose modifications: Cyclophosphamide and fludarabine will be dosed on actual body weight unless the participant has a body mass index (BMI) > 35. If the participant is obese (BMI > 35) drug dosage will be calculated using practical weight as described in **Appendix E**.

Fludarabine will be dose modified in participants with renal impairment. **In participants with a creatinine clearance calculated by the CKD-EPI equation < 80 mL/minute/1.73 m^2 of BSA, the daily dose of fludarabine will be reduced by 20%.**

Note: Chemotherapy infusions maybe slowed or delayed as medically indicated.

Supportive care:

1. Hydration and Mesna uroprotection

Begin hydration with 0.9% sodium chloride injection at 1.5 ml/kg/hour (max rate capped at 100 ml/hour) starting at least 6 hours pre-cyclophosphamide and continuing until 24 hours after last cyclophosphamide infusion. Furosemide 10-20 mg IV will be given once daily on cyclophosphamide treatment days to promote diuresis. At any time during the preparative regimen, if the urine output <1.5 ml/kg/hour or if body weight >2 kg over pre-cyclophosphamide value, additional doses of furosemide 10-20 mg IV may be administered. Intravenous hydration administered during cyclophosphamide will be individualized for participant clinical factors. Participants at risk of adverse clinical consequences from volume overload (e.g. participants with history of pulmonary hypertension or cardiac dysfunction) may be considered for low-dose hydration rates and/or volumes or hemorrhagic cystitis prevention strategies that include mesna alone without intravenous hydration.

Mesna 30 mg/kg/day will be administered by IV continuous infusion starting at approximately the same time as cyclophosphamide. The daily dose will be divided into two 12-hour infusion bags.

2. Antiemetic prophylaxis for chemotherapy induced nausea and vomiting

Antiemetics should be given prophylactically to prevent chemotherapy induced nausea and vomiting. A suggested regimen includes:

Ondansetron 0.15 mg/kg (*rounded to the nearest even mg dose between 8 mg and 16 mg based on participant weight*) IV every 8 hours x 3 days on Days -6, -5, and -4.

Aprepitant 125 mg PO one-time dose on Day -6 and Aprepitant 80 mg once daily x 2 doses on Days -5 and -4.

Olanzapine 5 to 10 mg PO once daily x 5 days starting on Day -6.

Modifications to the antiemetic regimen may be made based on clinical considerations but corticosteroids should be avoided.

3.2.2 Cell Infusion and other treatment Administration

The KK-LC-1 TCR T cells will be delivered to the participant care unit by an authorized staff member. Prior to infusion, the cell product identity label is double-checked by two authorized staff (MD or RN), an identification of the product and documentation of administration are entered in the participant's chart, as is done for blood banking protocols. See **Appendix G** on guidelines for management of infusion reactions.

Day 0 (two to four days after the last dose of fludarabine):

- KK-LC-1 TCR T cells will be administered intravenously over 20 to 30 minutes via non-filtered tubing, gently agitating the bag during infusion to prevent cell clumping.

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

- Fluconazole: can be used at the discretion of the treating clinician
- Valacyclovir po or Acyclovir IV, PO: will be administered until CD4 counts > 200/mm^3 X 2

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

- Aldesleukin as described in section **3.3** below.
- TMP/SMX may be administered at 160mg/800mg every other day at the time of count recovery

Dose 1-4 Schedule

Day	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**
Therapy											
Cyclophosphamide	X	X									

Day	-6	-5	-4	-3	-2	-1	0	1	2	3	4
30 mg/kg IV once daily x 2 days											
Ondansetron 0.15 mg/kg IV every 8 hours x 3 days	X	X	X								
Olanzapine 5-10mg PO once daily x 5 days	X	X	X	X	X						
Aprepitant 125 mg PO X1, 80 mg PO daily X2	X	X	X								
Mesna 30 mg/kg	X	X									
Fludarabine 25 mg/m^3 IV once daily x 5 days	X	X	X	X	X						
KK-LC-1 TCR cells							X				
Aldesleukin							X	X	X	X	X
Valacyclovir PO or Acyclovir IV, PO[7]							X	X	X	X	X

3.3 ALDESLEUKIN: INTRAVENOUS ADMINISTRATION

Aldesleukin will be administered at a dose of 720,000 IU/kg (based on total body weight) as an intravenous bolus over a 15 minute period beginning within 24 hours of cell infusion and continuing for up to four days (maximum 12 doses). The start of aldesleukin treatment may be delayed up to 3 days after cell infusion if medically necessary. Doses will be preferentially administered every eight hours; however, up to 24 hours may elapse between doses depending on participant tolerance. Aldesleukin dosing will be stopped if toxicities are not sufficiently recovered with supportive measures within 24 hours of the last dose of aldesleukin. Doses will be delayed or stopped if participants reach Grade 3 or 4 toxicity due to aldesleukin except for the reversible Grade 3 toxicities common to aldesleukin such as diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes as detailed in **Appendix C**. Toxicities will be managed as outlined in **Appendix B**. In addition, dosing may be held or stopped at the discretion of the treating investigator. Because confusion is a possible side effect of aldesleukin administration, a Durable

Power of Attorney will be signed by the participant to identify a surrogate to make decisions if a participant becomes unable to make decisions.

3.4 POTENTIAL REPEAT TREATMENT

Participants who were enrolled at a lower dose level may receive a second treatment course at the currently enrolling dose level. For instance, if a participant treated at dose level 1 progresses, and the study is currently enrolling participants at dose level 3, then the participant may receive a second treatment course at the currently enrolling higher dose level (in this case dose level 3). Participants must continue to meet the original eligibility criteria to be considered for retreatment. Research assessments will be performed at the same time intervals used for initial treatment. Participants who develop grade 3 or 4 toxicity due to cell infusion will not be retreated. A maximum of 1 retreatment course may occur. The second treatment will not begin prior to 6-8 weeks after the last dose of aldesleukin.

Note: Response data for all treatments will be captured in the database however only the response data from the first treatment will be used in the determination of response.

3.5 ON STUDY EVALUATION

Note: Refer to section **5** for research evaluations

3.5.1 Prior to starting the preparative regimen

- Apheresis as indicated
- Within 14 days prior to starting the preparative regimen, participants will have a complete blood count (CBC with differential), electrolytes, BUN, creatinine, liver function tests, TBNK, and serum chemistries performed. If any results are beyond the criteria established for eligibility, the treatment may be put on hold until the abnormalities can be resolved.

3.5.2 During the preparative regimen: (day -6 to cell infusion)

- Tests will be performed every 1-2 days, including Complete Blood Count (CBC with differential) Chemistries: Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, Total Protein
- Urinalysis (every 1-2 days)

3.5.3 After Cell Infusion

3.5.3.1 Daily (Day +1 to Day +7)

- Following cell administration - Vital signs will be monitored hourly (+/- 15 minutes) for four hours and then routinely (every 4-6 hours) unless otherwise clinically indicated
- CBC
- Chemistries: Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin

- Once total lymphocyte count is greater than 200/mm^3, TBNK for peripheral blood CD4 count will be drawn weekly (within one business day, while the participant is hospitalized)
- Other tests may be performed as clinically indicated including Calcium total, Magnesium total (Mg), Phosphorus, LD, Total Protein, Total CK, Uric Acid

3.5.4 During Hospitalization (approximately 14-21 days)

3.5.4.1 Every 1-2 days

- A review of systems and physical exam as clinically indicated
- Tests may be performed as clinically indicated including Complete Blood Count (CBC with differential), Chemistries: Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid
- Once total lymphocyte count is greater than 200/mm^3, TBNK for peripheral blood CD4 count will be drawn weekly Monday through Thursday (within one business day, while the participant is hospitalized).
- KK-LC-1 TCR assay may be collected 1-2 times per week
- Surveillance blood cultures may be drawn at the discretion of the investigator and continue as clinically indicated.
- Other tests will be performed as clinically indicated.

3.6 POST TREATMENT EVALUATION (FOLLOW-UP)

At each scheduled safety and response evaluation, participants will undergo:

a. Physical examination

b. Chemistries: Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, Total Protein

c. Complete blood count

d. Thyroid panel as clinically indicated

e. TBNK, until CD4 > 200/mm^3 x 2

f. Toxicity assessment, including a review of systems

g. CT of the chest, abdomen and pelvis as clinically indicated. If clinically indicated, other scans or x-rays may be performed, e.g. PET, brain MRI, bone scan

h. KK-LC-1 TCR Assay

i. An approximately 5 liter apheresis may be performed at the first follow up visit. If the subject is unable to undergo apheresis, approximately 96 ml of blood may be obtained. Subsequently, approximately 60 ml of blood will be obtained at follow up visits for at least three months. Peripheral blood mononuclear cells will be cryopreserved so that

immunologic testing may be performed and will be banked under protocol 16C0061 (Tissue Procurement Protocol). PBMC from apheresis will be stored in 10 vials at $100x10^6$ cells per vial with remaining cells stored at $300x10^6$ cells per vial.

3.6.1 Stable Disease, Partial Response, Complete Response or Unresolved Toxicities

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

- Week 12 (+/- 2 weeks)
- Every 3 months (+/- 1 month) x3
- Every 6 months (+/- 1 month) x 5 years
- As per PI discretion for subsequent years

Note: Participants may be seen more frequently as clinically indicated

3.6.2 Long-Term Follow-up

Long-term follow up of participants receiving gene transfer is required by the FDA and must continue even after the participant comes off the study. Long-term follow-up will be performed as indicated in the long term gene therapy follow-up protocol (20C0051) and participants will be co-enrolled. A baseline (pre-treatment) sample will be collected on protocol 20C0051 to improve analysis of the long term follow up testing.

After the participant comes off study, health status data will be obtained from surviving participants via telephone contact or mailed questionnaires for a total of 15 years after cell infusion. Refer to section **5.3**.

3.7 STUDY CALENDAR

For the treatment schedule, please refer to Section **3.2.2**.

Note: The retreatment schedule (see Section **3.4**) will be identical to the schedule for participants being treated for the first time, including eligibility assessments. A maximum of one course of treatment will be allowed on retreatment.

Procedure	Screening/ Baseline[2]	Before treatment	Preparative regimen[5]	Day 0	During hospitalization[6]	Follow-up[9] (End of Treatment)
Medical history	X					
Physical exam	X			X	X	X
Confirmation of Diagnosis (KK-LC-1 positivity assay), Rutgers Cancer Institute	X					
ECOG performance status	X					
NIH Advance Directives Form[16]						
Chest x-ray	X					
EKG	X					
CT and MRI or PET	X					X
Pulmonary Function Test[1]	X					
Cardiac evaluation[10]	X					
Viral titers	X					
Optional Biopsy	X				X[12]	
Blood chemistries[8]	X	X	X		X	X
Complete blood count (CBC with diff.)	X	X	X		X	X
Thyroid panel	X					X[13]
HLA typing	X					
TBNK		X			X[7]	X
Urinalysis	X		X			
Pregnancy test[3]	X					
Leukapheresis		X[17]				X[15]
Infusion of transduced cells[4]				X		
Additional apheresis or blood draw[11]						X
Research blood	X	X		X[14]		X
KK-LC-1 TCR assay[18]					X	X

1. For participants with a prolonged history of cigarette smoking, as indicated in Section **2.3.2.3**
2. Exact timeline is indicated in Sections **2.3** and **2.5**
3. For women of child-bearing potential as defined in Section **2.1.1.10**
4. See other treatments in Schedules, Section **3.2.2**
5. On days -6 to -1, every 1 -2 days as clinically indicated
6. Every 1 to 2 days while hospitalized
7. Once total lymphocyte count is greater than 200/mm^3, TBNK for peripheral blood CD4 count will be drawn weekly (within one business day, while the participant is hospitalized)
8. Chemistries Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, Total Protein
9. End of treatment visit will occur approximately 40 days (+/- 2 weeks) after cell infusion, additional visits as indicated in Section **3.6.1**
10. For participants who are greater than or equal to 50 years of age, or who have a history of ischemic heart disease, chest pain or clinically significant atrial and/or ventricular arrhythmias. Participants with a LVEF of less than or equal to 45% will not be eligible, as noted in section **2.3.2**
11. As described in section **3.6**.
12. Following treatment (6 weeks post treatment preferred) and at disease progression.
13. As clinically indicated
14. Monday, Wednesday, Friday during hospitalization once ALC > 200/mm^3
15. If the participant is unable to undergo apheresis, approximately 96 ml of blood may be obtained. Apheresis will only be done in the first follow-up visit; in the following visits, blood will be collected.
16. All subjects will be offered the opportunity to complete an NIH advanced directives form. This should be done preferably at baseline but can be done at any time during the study as long as the capacity to do so is retained. The completion of the form is strongly recommended but is not required.
17. This can occur at any time prior to treatment on protocol 16C0061 if the participant is co-enrolled on that protocol. See Section **3.1.1** for further details. For participants undergoing retreatment, retreatment will only occur if there are enough cells stored from their prior course. See Section **3.4** for details on retreatment.
18. Clinical assay performed by the NCI Flow Cytometry Laboratory in the Laboratory of Pathology. The assay may be collected at follow-up visits

3.8 COST AND COMPENSATION

3.8.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures are performed outside the NIH Clinical Center, participants may have to pay for these costs.

3.8.2 Compensation

No compensation is provided for participation in this study.

3.8.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.9 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 40 days following cell infusion.

3.9.1 Criteria for removal from protocol therapy

Participants will be taken off treatment for the following:

- Completion of treatment
- Grade 3 or greater autoimmunity that involves vital organs (heart, kidneys, brain, eye, liver, colon, adrenal gland, lungs).
- If a subject experiences Grade 3 or higher toxicity due to cell infusion (reaction to cellular product or infusion reaction), the participant will receive no further treatment.
- Participant requests to be withdrawn from active therapy
- Investigator Discretion
- Positive pregnancy test

3.9.2 Off-Study Criteria

Participants will be taken off study for the following:

- Screen failure
- The participant voluntarily withdraws
- There is significant noncompliance
- Progressive disease
- Death
- Study closure
- Completion of study follow-up period
- Participant lost to follow-up
- Investigator discretion

Note: Participants who are taken off study for study closure may be followed on the Long-Term Gene Therapy Follow-up Protocol (20C0051).

3.9.3 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for 3 scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 1-2 days and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, an IRB approved certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 PROHIBITED MEDICATIONS

Current use of immunosuppressive medication, EXCEPT for the following:

- Intranasal, inhaled, topical steroids, or local steroid injection (e.g., intra-articular injection)
- Systemic corticosteroids at physiologic doses ≤ 10 mg/day of prednisone or equivalent; or,
- Steroids as premedication for hypersensitivity reactions (e.g., CT scan premedication)

4.2 INFECTION PROPHYLAXIS

Note: Other medications may be substituted or held at the discretion of the treating investigator. Below are guidelines and suggested medications and schedule to be used, however they can be altered by the treating physician as clinically indicated.

4.2.1 Pneumocystis Jirovecii Pneumonia

Participants may receive the fixed combination of trimethoprim and sulfamethoxazole [SMX] as double strength (DS) tab (DS tabs = TMP 160 mg/tab, and SMX 800 mg/tab) P.O. daily three times a week on non-consecutive days at the time of count recovery.

Pentamidine may be substituted for TMP/SMX-DS in participants per investigator discretion. It will be administered aerosolized at 300 mg per nebulizer approximately within one week of chemotherapy start date.

4.2.2 Herpes Virus Prophylaxis

Participants may be given either acyclovir 800mg PO twice daily (preferred) or valacyclovir 500mg PO daily (alternate) or, if unable to tolerate PO: acyclovir 250mg/m2 IV q 12 hr. Reversible renal insufficiency has been reported with IV but not oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs have been reported with higher doses of acyclovir. Should this occur, a dosage adjustment will be made or the drug will be discontinued. Acyclovir will not be used concomitantly with other nucleoside analogs which interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling.

Prophylaxis for Pneumocystis and Herpes will continue for 6 months post chemotherapy. If the CD4 count is less than 200 at six months post chemotherapy, prophylaxis will continue for at least six months and until the CD4 count is greater than 200 for two consecutive measures.

4.2.3 Fungal Prophylaxis (Fluconazole)

Participants may start Fluconazole 400 mg po. starting on the day of cell infusion and continue until the absolute neutrophil count is greater than 1000/mm^3. The drug may be given IV at a dose of 400 mg in 0.9% sodium chloride USP daily in participants unable to take it orally.

4.2.4 Empiric Antibiotics

Participants will start on broad-spectrum antibiotics as per 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/mm^3. Infectious disease consultation will be obtained for all participants with unexplained fever or any infectious complications.

4.2.5 Blood Product Support

Using daily CBC's as a guide, the participant will receive platelets and packed red blood cells (PRBC's) as needed. As a general guideline, participants may be transfused for:

- Hemoglobin < 8 gm/dl
- Platelets < 10,000/mm^3

Note: Participants may be transfused at a higher platelet count as clinically indicated, e.g.:

  - Increased risk for bleeding such as undergoing an invasive procedure or presence of metastatic lesion likely to bleed
  - fever greater than 38.5°C
  - sepsis

All blood products will be irradiated. Leukocyte filters will be utilized for all blood and platelet transfusions to decrease sensitization to transfused WBC's and decrease the risk of CMV infection.

4.2.6 Neutrophil Recovery

Participants may receive Filgrastim (or filgrastim biosimilar) for count recovery when clinically indicated per national guidelines.

4.3 OTHER CONCOMITANT MEDICATIONS TO CONTROL SIDE EFFECTS

Concomitant medications to control side effects of therapy may be given. Meperidine (25-50 mg) may be given intravenously if severe chilling develops. Other supportive therapy may be given as required and may include acetaminophen (650 mg q4h), indomethacin (50-75 mg q8h) and famotidine (20 mg g12h). If participants require steroid therapy they will be taken off treatment. Participants who require transfusions will receive irradiated blood products. Ondansetron 0.15 mg/kg/dose IV every 8 hours may be administered for nausea and vomiting. Additional anti-emetics may be administered as needed for nausea and vomiting uncontrolled by ondansetron. Antibiotic coverage for central venous catheters may be provided at the discretion of the investigator.

5 CORRELATIVE STUDIES FOR RESEARCH

5.1 BIOSPECIMEN COLLECTION

The amount of blood that may be drawn from adult participants for research purposes shall not exceed 10.5 mL/kg or 550mL; whichever is smaller, over any 8-week period.

Note: Please note that tubes and media may be substituted based on availability with the permission of the PI or laboratory investigator.

5.1.1 Pre-cell infusion evaluations

- At baseline/screening 12 CPT tube and 2 SST tubes may be collected starting on the day of chemotherapy. One CPT tube may be used to collected 4mL of plasma, which may be frozen in 4mL vials. PBMC from the remainder of the CPT tubes may be frozen in aliquots of 10 x 10^6 cells/vial. Serum from SST tubes may aliquoted into four vials of 0.5-1mL each. All samples will be processed in the Blood Processing Core (BPC). All baseline/screening blood research specimens will be collected.
- At day -6 prior to cell infusion 6 CPT tubes and 1 SST tube may be collected starting on the day of chemotherapy. One CPT tube may be used to collect 4mL of plasma, which may be frozen in 4mL vials. PBMC from the remainder of the CPT tubes may be frozen in aliquots of 10 x 10^6 cells/vial. Serum from SST tubes may be aliquoted into four vials of 0.5-1mL each. All samples will be processed in the Blood Processing Core (BPC).

5.1.2 Post cell infusion evaluations

- 2 SST tubes (4mL) may be collected daily for serum starting on the day of chemotherapy and continuing through the end of hospitalization. Serum will be processed in the Blood Processing Core (BPC) and may be aliquoted into four vials of 0.5-1 mL each.
- Once total lymphocyte count is greater than 200/mm^3, the following samples may be drawn and sent to the Blood Processing Core (BPC) on Monday, Wednesday, and Friday x 5 days, then weekly (while the participant is hospitalized). Send to the Blood Processing Core (BPC). Attention: NCIBloodcore@mail.nih.gov. Building 10, room 5A08 pager is 11964, tel # 240 760 6180.
    - 6 CPT tubes (8mL each). One CPT tube daily may be used to collect 4mL of plasma, which may be frozen in 4mL vials. PBMC from the remainder of the CPT tubes may be frozen in aliquots of 10 x 10^6 cells/vial

- Following discharge, at each scheduled follow-up visit 6 CPT tubes and 1 SST tube may be collected. One CPT tube may be used to collect 4mL of plasma, which may be frozen in 4mL vials. PBMC from the remainder of the CPT tubes may be frozen in aliquots of 10 x 10^6 cells/vial. Serum from SST tubes may be aliquoted into four vials of 0.5-1mL each. Samples will be processed in the Blood Processing Core (BPC).

5.1.3 Tumor Biopsies

- Tumor may be biopsied pre- and post-treatment in order to test for KK-LC-1 expression, HLA expression, and other characteristics that may affect response to therapy. Biopsies are not required; a maximum of three biopsies, with three cores/attempts, may be performed.
- Tissue may be obtained pre-treatment, following treatment (6 weeks post treatment preferred) and at the time of progression. Biopsies will only be performed at the NCI CCR.
- Tissue may be obtained via CT, MRI or US guided biopsy under IV (conscious) sedation as appropriate.
- Specimens may be transported by the assigned research nurse to the CIO cellular therapy lab for sample labeling. Contact: Scott Norberg, Bldg 10, room 4B-04, phone 301-275-9668. Following labeling, samples may be transported by an assigned lab member to the Blood Processing Core (BPC) where they will be stored. Samples may also be transported to and labeled by BPC. Contact: Blood Processing Core (BPC) Attention: NCIBloodcore@mail.nih.gov, Building 10, room 5A08 pager is 11964, tel # 240 760 6180.
- Some of these samples may be archived and analyzed under another protocol 16C0061 (Tissue Procurement Protocol) if the subject is also enrolled on that study.

5.1.4 Immunological Testing

- Apheresis may be performed prior to and about four to six weeks after the treatment. Apheresis product will be transferred to the Blood Processing Core (BPC) Attention: NCIBloodcore@mail.nih.gov. Building 10, room 5A08 pager is 11964, tel # 240 760 6180. Cell product may be frozen in 10 vials at concentration 100 x 10^6 cells/mL and additional vials at 300 x 10^6 cells/mL.
- At other time points, peripheral blood lymphocytes (PBL) and plasma may be obtained from whole blood by purification using centrifugation. These samples may be transferred directly to the Blood Processing Core (BPC) lab for processing. Plasma may be frozen in 4mL vials. PBL may be frozen in aliquots of 10 x 10^6 cells/vial
- Possible laboratory research studies on tumor biopsies are as follows: Expression of CD3, CD4, CD8, MHC I, and MHC II by immunohistochemistry; flow cytometry to determine the frequency of KK-LC-1 TCR T cells in the samples; generation and characterization of TIL cells; generation and characterization of tumor cell lines. IHC quantification may be by blinded scoring of the intensity and frequency of staining. Flow cytometry data will be analyzed with FlowJo software. Biopsies - will only be performed if minimal morbidity is expected based on the procedure performed.

- Possible laboratory research studies on PBMC and PBL are as follows: Specific cytolysis determined by impedance-based assay, frequency of effector cells as determined by ELISPOT, quantity of cytokine production as determined by coculture assay with cytokine quantification, cytokine production by intracellular flow cytometry, phenotypic analysis by flow cytometry. Immunological assays may be standardized by the inclusion of 1) pre-infusion PBMC and 2) an aliquot of the T cells cryopreserved at the time of infusion.
- Possible laboratory research on serum or plasma are as follows: cell free DNA quantification, cytokine quantification
- The planned methods for performing the laboratory studies above are as described in Stevanovic, et al, *Journal of Clinical Oncology*, 2015 and Draper, et al, Clinical Cancer Research, 2015. [42, 51]
- The laboratory studies are considered exploratory. Statistical analysis will be performed in consultation with a biostatistician.
- Specimens collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study and protocol 16C0061 (Tissue Procurement Protocol).

5.1.5 Samples/Data sent to Rutgers Cancer Institute of New Jersey

KK-LC-1 testing will be performed via an IHC assay developed by Rutgers Cancer Institute of New Jersey. This testing will be performed on archival samples or on fresh tissue that is collected at screening for confirmation of diagnosis.

The data may include demographic and clinical outcome information. Samples and data will be deidentified. Identifiable information will only be provided, if needed, to interpret data.

5.2 SUMMARY OF SAMPLE COLLECTION

Test/assay	**Volume blood (approx)**	**Type of tube[a]**	**Collection point (+/- 48hrs)**	**Location of specimen analysis**
Plasma/ PBMC	48-96 mL	CPT	<4 weeks prior to cell infusion, day-6	Blood Processing Core (BPC)
Serum	8-16 mL	SST	<4 weeks prior to cell infusion, day-6	Blood Processing Core (BPC)
Plasma/ PBMC	48 mL	CPT	Post cell infusion day 1, 3, every Mon/Wed/Fri x 5 days, weekly until discharge and follow-up visits	Blood Processing Core (BPC)

Test/assay	Volume blood (approx)	Type of tube[a]	Collection point (+/- 48hrs)	Location of specimen analysis
Serum	Variable, based on length of hospitalization and duration of follow-up	SST	Daily at the start of chemo until discharge from hospital, follow-up visits	Blood Processing Core (BPC)
Optional Biopsy	N/A	N/A	Prior to starting chemotherapy, at the first response assessment visit and at the time of progression	Transported and processed in CIO cellular therapy lab or Blood Processing Core (BPC)
a. Please note that tubes and media may be substituted based on availability with the permission of the PI or laboratory investigator.				

5.3 GENE-THERAPY-SPECIFIC FOLLOW-UP

Persistence of TCR transduced cells will be assessed by quantitative PCR and/or flow cytometry at follow-up visits 1, 3, 6 and 12 months after cell infusion, or until TCR-expressing cells are no longer detectable. Participants' blood samples will be obtained and undergo analysis for detection of replication competent retroviruses (RCR) by PCR prior to cell infusion, and at 3, 6, and 12 months post cell administration, and annually thereafter for 4 more years. RCR testing will continue until three negative consecutive tests results are obtained. These cells will be obtained from the CPTs drawn for research at follow up visits or under the long-term gene therapy follow up protocol if the participant is off study.

5.4 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

5.4.1 Samples Managed by the Blood Processing Core (BPC)

5.4.1.1 Blood Collection

Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov

The samples will be processed, barcoded, and stored in the BPC lab until requested by the investigator.

5.4.1.2 Sample Data Collection

All samples sent to the Blood Processing Core (BPC) will be barcoded, with data entered and stored in Labmatrix utilized by the BPC. This is a secure program, with access to Labmatrix limited to defined BPC lab personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by the BPC. These computers all have a password restricted login screen.

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to participants without Labmatrix access. The data recorded for each sample includes the participant ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Participant demographics associated with the clinical center participant number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.4.2 CIO Cellular Therapy Laboratory

Samples transferred to the CIO cellular therapy laboratory may be barcoded and tracked with Labmatrix.

Laboratory research data will be stored on the NCI secure server with secure access by laboratory personnel only. Access to personally identifiable information (PII) is limited to the PI and study personnel who interact directly with the participant and their samples.

5.4.3 Sample Storage and Destruction

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in Labmatrix. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a participant withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the participant, if so requested). The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.1**.

Sample barcodes are linked to participant demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the

Labmatrix. It is critical that the sample remains linked to participant information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The NIH PI will be responsible for overseeing entry of data into a 21 CFR Part 11-compliant data capture system provided by the NCI CCR and ensuring data accuracy, consistency and timeliness. The Principal Investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

The key for assignment of participant code identification numbers with the personal identifiers will be stored in a secure data base. This key will not be shared with other investigators. Investigators conducting the individual sample testing will only have access to coded identification numbers and coded participant information (i.e. treatment regimens, treatment responses, diagnoses, pathology information).

Data from analyses performed at participating site will be provided to the NIH team by secure email and entered into the study database.

6.1.1 Adverse Event (AE) Recording

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. All study related adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of attribution will be captured in study database up to 40 days following last administration of investigational agent. AEs will be documented from the start date of chemotherapy through 40 days after the cell administration. Beyond 40 days after the cells were administered, only adverse events which are serious and related to the study intervention need to be recorded.

In addition, all incidences of intubation including the duration of and reason for intubation will be captured in study database.

6.1.2 Reporting of Laboratory Events

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

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

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

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

6.2.2 What data will be shared?

I will share human data generated in this research for future research as follows:

- Coded, linked data in an NIH-funded or approved public repository.
- Coded, linked data in BTRIS (automatic for activities in the Clinical Center)
- Coded, linked or identified data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

Data will be shared through:

- An NIH-funded or approved public repository: ClinicalTrials.gov.
- BTRIS (automatic for activities in the Clinical Center)
- Approved outside collaborators under appropriate individual agreements.
- Publication and/or public presentations.

When will the data be shared?

- Before publication.
- At the time of publication or shortly thereafter.

6.3 RESPONSE CRITERIA

For the purposes of this study, participants should be re-evaluated for response as indicated in Section **3.6.1**. In addition to a baseline scan, confirmatory scans should also be obtained 4 to 8 weeks following initial documentation of objective response.

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) for participants with solid tumors [58]. In cases where participants have isolated bony metastases that do not meet RECIST criteria, Positron Emission Tomography Response Criteria in Solid Tumors (PERCIST) (version 1.0) guidelines will be used [59]. 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. Normalization of standardized uptake value on FDG-PET is used in PERCIST. For participants that have both bony lesions and lesions that meet RECIST criteria, only RECIST criteria will be used to evaluate best overall response.

6.3.1 Definitions

Evaluable for toxicity: All participants will be evaluable for toxicity from the time of their first treatment with KK-LC-1 TCR transduced PBL.

Evaluable for objective response: Only those participants who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These participants will have their response classified

according to the definitions stated below. (Note: Participants who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response: Participants who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

6.3.2 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as:

- By chest x-ray: ≥ 20 mm;
- By CT scan:
    - Scan slice thickness 5 mm or under: as ≥ 10 mm
    - Scan slice thickness > 5 mm: double the slice thickness
- With calipers on clinical exam: ≥ 10 mm.

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Measurable bone metastases: Any size bone lesions with a baseline SUL (standard uptake value, corrected for lean body mass) on FDG-PET of 1.5 x mean liver SUL + 2 standard deviations of mean SUL is considered measurable. Bone lesions are only measured in participants with isolated bone metastases.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease. Lesions that have been previously treated with loco-regional therapy

or in a previously irradiated area can be considered target lesions if they have demonstrated unequivocal progression by radiographic imaging.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

6.3.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray: Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

FDG-PET: Participants with isolated bony metastases will have FDG-PET to determine if lesions meet criteria to be followed by PERCIST version 1.0. While FDG-PET response assessments in solid tumors being followed by RECIST need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression of lesions (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of

PD based on a new lesion.

b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers: Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a participant to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published. [60-62] I In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer.[63]

Cytology, Histology: These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

6.3.4 Response Criteria

6.3.4.1 Evaluation of Target 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.

6.3.4.2 Evaluation of Bony Lesions in Participants 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.

6.3.4.3 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 participant 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).

6.3.4.4 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 participant's best response assignment will depend on the achievement of both measurement and confirmation criteria.

6.3.5 For Participants with Measurable Disease (i.e., Target Disease)

Target Lesions	**Non-Target Lesions**	**New Lesions**	**Overall Response**	**Best Overall Response when Confirmation is Required***
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. 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 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion. ** Only for non-randomized trials with response as primary endpoint. *** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression. Note: Participants 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 Participants with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-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 an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

6.3.6 Duration of Response

Duration of overall response: The duration of overall response is measured from the time of cell infusion 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.

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each participant while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

7 NIH REPORTING REQUIREMENTS / DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements. Note: Only IND Safety Reports that meet the definition of an unanticipated problem or present new information that might affect the willingness of participants to enroll or remain on the study will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reviewed by the OHSRP in the NIH eIRB system will also be reported to the NCI Clinical Director/designee; therefore, a separate submission for these reports is not necessary.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 NCI GUIDANCE FOR REPORTING EXPEDITED ADVERSE EVENTS FOR MULTI-CENTER TRIALS

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802: Non-compliance in Human Subjects Research, found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements. The participating site PI must

immediately report to the coordinating center PI any deaths possibly related to the research within 24 hours of PI awareness of the event. The Site PI must also report any other events required by Policy 801 to the coordinating center PI within 7 days of PI awareness.

A reporting form will be provided with the participating site documents for such reports.

7.5 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

7.5.1 Serious Adverse Event Reports to IBC

The NIH Principal Investigator (or delegate) will notify IBC of any unexpected fatal or life-threatening experience associated with the use of KK-LC-1 TCR T cells as soon as possible but in no event later than 7 calendar days of initial receipt of the information. Serious adverse events that are unexpected and associated with the use of the KK-LC-1 TCR T cells, but are not fatal or life-threatening, must be reported to the NIH IBC as soon as possible, but not later than 15 calendar days after the investigator's initial receipt of the information. Adverse events may be reported by using the FDA Form 3500a.

7.5.2 Annual Reports to IBC

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

7.5.3 Clinical Trial Information

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

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

7.5.4 Progress Report and Data Analysis

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

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

7.6 NIH Required Data and Safety Monitoring Plan

7.6.1 Principal Investigator/Research Team

The clinical research team will meet on a regular biweekly basis when participants are being actively treated on the trial to discuss each participant. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior participants.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in section **7.2.1** will be submitted within the appropriate timelines.

The NIH principal investigator will review adverse event and response data on each participant to ensure safety and data accuracy. The site principal investigator will personally conduct or supervise the investigation at the respective site and provide appropriate delegation of responsibilities to other members of the research staff.

7.6.2 Safety Monitoring Committee (SMC) – NCI CCR SMC

This protocol will be periodically reviewed by an intramural Safety Monitoring Committee. comprising physicians, biostatisticians and a lay member selected based on experience, area of expertise, reputation for objectivity, absence of conflicts of interest and knowledge of or experience with clinical trial research. Initial review will occur as soon as possible after the annual NIH Intramural IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC based on the risks presented in the study. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period.

The SMC will operate under the rules of an approved charter that will be written and reviewed at the organization meeting of the SMC. Each review will focus on unexpected protocol-specific safety issues that are identified during the conduct of the clinical trial.

Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal Investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

8 SPONSOR PROTOCOL/SAFETY REPORTING

8.1 DEFINITIONS

8.1.1 Adverse Event

Any untoward medical occurrence in a participant or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2))

8.1.2 Serious Adverse Event (SAE)

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

- Death
- A life-threatening adverse event (see section **8.1.3**)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for participant or subject convenience) is not considered a serious adverse event.
    - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the participant or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the participant or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32)

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 5.0.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 Assessment of Safety Events

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to section **6.1.16.1**. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor with the exception of any listed in section **8.4**.

8.3 Reporting of Serious Adverse Events

Any AE that meets protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form. Any exceptions to the expedited reporting requirements are found in section **8.4**.

All SAE reporting must include the elements described in section **8.2**.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 WAIVER OF EXPEDITED REPORTING TO CCR

As the conditioning regimen is known to result in Hematological toxicities, only those SAEs that are not specified in the table below will be reported to the Sponsor in an expedited manner.

CTCAE System Organ Class	Adverse Event	Grade	Prolongation of Hospitalization	Expected Frequency	Attribution
Investigations	Neutrophil count decreased	1-3	Expected	100%	Commercial Product (Cyclophosphamide and fludarabine)
Investigations	Neutrophil count decreased	4 if < 14 days	Expected	100%	Commercial Product (Cyclophosphamide and fludarabine)
Blood and lymphatic system disorders	Anemia	1-3	Expected	100%	Commercial Product (Cyclophosphamide and fludarabine)
Investigations	Platelet count decreased	1-3	Expected	100%	Commercial Product (Cyclophosphamide and fludarabine)
Investigations	Platelet count decreased	4 if < 14 days	Expected	100%	Commercial Product (Cyclophosphamide and fludarabine)
Investigations	White blood cell decreased	1-4	Expected	100%	Commercial Product (Cyclophosphamide and fludarabine)
Investigations	Lymphocyte count decreased	1-4	Expected	100%	Commercial Product (Cyclophosphamide and fludarabine)
Investigations	CD4 lymphocytes decreased	1-4	Expected	100%	Commercial Product (Cyclophosphamide and fludarabine)

Blood and lymphatic system disorders	febrile neutropenia (without an associated infection)	3	Expected	100%	Commercial Product (Cyclophosphamide and fludarabine)

The PI will submit a summary table of all grade 3-5 events, whether or not considered related to the product, every 6 months. The report shall include the number of participants treated in the timeframe, the number of events per AE term per grade which occurred in the 6-month timeframe and in total since the start of the study, attribution, and type/category of serious.

Reports will be submitted to the Center for Cancer Research (CCR) at OSROSafety@mail.nih.gov

The Sponsor might request case summaries for those events if, upon review, the Sponsor determines that an aggregate safety report is required (21CFR312.32(c)(1)(iv)).

8.5 Safety Reporting Criteria to the Pharmaceutical Collaborators

Reporting will be per the collaborative agreement with T-Cure Bioscience (CRADA #03313)

8.6 Reporting Pregnancy

All required pregnancy reports/follow-up to OSRO will be submitted to: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. Forms and instructions can be found here: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

8.6.1 Maternal exposure

If a participant becomes pregnant during the course of the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy becomes known,

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (section **8.1.2**) should be reported as SAEs.

The outcome of all pregnancies should be followed up and documented.

8.6.2 Paternal exposure

Participants should refrain from fathering a child or donating sperm during the study and for up to 4 months after the last dose of KK-LC-1 TCR T cells.

Pregnancy of the participant's partner is not considered to be an AE. The outcome of all pregnancies occurring from the date of the first dose until 4 months after the last dose should, if possible, be followed up and documented. Pregnant partners may be offered the opportunity to participate in an institutional pregnancy registry protocol (e.g., the NIH IRP pregnancy registry study) to provide data about the outcome of the pregnancy for safety reporting purposes.

8.7 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected in expediated manner to the FDA in accordance to 21 CFR 312.32. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

8.8 SPONSOR PROTOCOL DEVIATION REPORTING

A Protocol Deviation is defined as any non-compliance with the clinical trial Protocol, Manual of Operational Procedures (MOP) and other Sponsor approved study related documents, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the study site staff inclusive of site personnel performing procedures or providing services in support of the clinical trial.

It is the responsibility of the study Staff to document any protocol deviation identified by the Staff or the site Monitor in the CCR Protocol Deviation Tracking System (PDTS) online application. The entries into the PDTS online application should be timely, complete, and maintained per CCR PDTS user requirements.

> Non-NIH participating sites not using the CCR Protocol Deviation Tracking System (PDTS) will report any protocol deviation on the OSRO Site Protocol Non-Adherence/Deviation Log, or a site-generated protocol deviation report approved by OSRO. The Non-Adherence/Deviation Log should be maintained in the site essential documents file and submitted to OSRO via OSROMonitoring@mail.NIH.gov on the **first business day of each month throughout the study**.

In addition, any deviation to the protocol should be documented in the participant's source records and reported to the reviewing IRB per their guidelines. OSRO required protocol deviation reporting is consistent with E6(R2) GCP: Integrated Addendum to ICH E6(R1): 4.5 Compliance with Protocol; 5.18.3 (a), and 5.20 Noncompliance; and ICH E3 16.2.2 Protocol deviations.

9 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure:

- that the rights of the participants are protected;
- that the study is implemented per the approved protocol, Good Clinical Practice and standard operating procedures;
- that the quality and integrity of study data and data collection methods are maintained.

Monitoring for this study will be performed by NCI CCR Office of Sponsor and Regulatory Oversight (OSRO) Sponsor and Regulatory Oversight Support (SROS) Services contractor. Clinical site monitoring activities will be based on OSRO standards, FDA Guidance E6(R2)

Good Clinical Practice: Integrated Addendum to ICH E6(R1) March 2018, and applicable regulatory requirements.

Details of clinical site monitoring will be documented in a Clinical Monitoring Plan (CMP) developed by OSRO. CMPs will be protocol-specific, risk-based and tailored to address human subject protections and integrity of the study data. OSRO will determine the intensity and frequency of monitoring based on several factors, including study type, phase, risk, complexity, expected enrollment rate, and any unique attributes of the study and the site. The Sponsor will conduct a periodic review of the CMP to confirm the plan's continued appropriateness. A change to the protocol, significant or pervasive non-compliance with GCP, or the protocol may trigger CMP updates.

OSRO SROS Monitoring visits and related activities will be conducted throughout the life cycle of each protocol. The first activity is before the study start to conduct a Site Assessment Visit (SAV) (as warranted), followed by a Site Initiation Visit (SIV), Interim Monitoring Visit(s) (IMVs), and a study Close-Out Visit (COV).

Some monitoring activities may be performed remotely, while others will occur at the study site(s). Monitoring visit reports will describe visit activities, observations, and associated action items or follow-up required for resolution of any issues, discrepancies, or deviations. Monitoring reports will be distributed to the study PI, NCI CCR QA, CCR Protocol Support Office, coordinating center (if applicable) and the Sponsor regulatory file.

The site Monitor will inform the study team of any deviations observed during monitoring visits. If unresolved, the Monitor will request that the site Staff enter the deviations in the CCR Protocol Deviation Tracking System (PDTS) for deviation reporting to the Sponsor and as applicable per institutional and IRB guidance.

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL HYPOTHESIS

10.1.1 Primary Objective

To determine the maximally tolerated dose of KK-LC-1 TCR T cells plus aldesleukin for the treatment of metastatic KK-LC-1 positive epithelial cancers.

10.1.2 Secondary objectives

None

10.1.3 Exploratory Objective(s)

To assess the safety and efficacy of KK-LC-1 TCR T cells plus aldesleukin for the treatment of KK-LC-1+ cancers

To conduct exploratory immunologic studies to understand and improve the administered treatment.

10.2 SAMPLE SIZE DETERMINATION

The protocol will enroll one participant per dose level, unless the participant has a DLT, in which case 6 participants will be treated at the dose level with subsequent escalation only if the remaining 5 participants do not have a DLT (1/6 total has a DLT at the dose level). Then, the

trial will enroll up to 6 participants per dose level for the remaining dose levels if there is no more than 1 participant with a DLT per dose level. With up to 6 dose levels explored and 6 more participants at the MTD, the maximum number of participants who could be treated will be 36, but as few as 5 (6 dose levels x 1 participant per level) +6 at the MTD plus another 6 at the MTD equals 17 participants if there is no more than minimal toxicity up through dose level 5, and 6+6=12 participants are enrolled at the MTD or highest safe dose level.

As indicated in section **3.4**, participants may be re-enrolled on the study as new participants to allow retreatment, and these participants will be considered both times in the total accrual ceiling for the study. Response data for all treatments will be captured in the database however only the response data from the first treatment will be used in the determination of response.

It is expected that up to 10 evaluable participants could be accrued onto this trial per year; accrual could be completed within 2-3 years. With up to 30 evaluable participants needed for treatment, as well as planning for a small number of inevaluable participants (5), we intend to initiate intervention in up to 35 participants. Note: To allow for screen failures (65), a total of 100 will be set for the purposes of the NIH accrual ceiling.

10.3 Populations for Analyses

10.3.1 Evaluable for toxicity

All participants receiving at least one dose of cells will be evaluable for toxicity from the time of their first treatment.

10.3.2 Evaluable for Objective Response

Only those participants who have measurable disease present at baseline, have received at least one course of therapy, and have had their disease re-evaluated will be considered evaluable for response. Participants who exhibit progression before disease can be re-evaluated will also be considered as part of the denominator for the fraction of participants who have responded.

10.4 Statistical Analyses

10.4.1 General Approach

The toxicities experienced by participants at each dose level will be reported per dose level.

10.4.2 Measures to Minimize Bias: Randomization and Blinding

None are used.

10.4.3 Analysis of the Primary Endpoints

The toxicities experienced by participants at each dose level will be reported per dose level. The grade as well as the type of toxicity will be tabulated per dose level. The fraction of participants who experience a DLT will be identified at a given dose level, with information reported about the number and grade of each type of DLT identified.

10.4.4 Analysis of the Secondary Endpoint(s)

None

10.4.5 Safety Analyses

The toxicities experienced by participants at each dose level will be reported per dose level. The grade as well as the type of toxicity will be tabulated per dose level. The fraction of participants

who experience a DLT will be identified at a given dose level, with information reported about the number and grade of each type of DLT identified.

10.4.6 Baseline Descriptive Statistics

Standard sample statistics will be reported for participants enrolled on the trial.

10.4.7 Planned Interim Analyses

Toxicities will be evaluated at each dose level to establish the ability to accrue participants onto the next dose level.

10.4.8 Sub-Group Analyses

None

10.4.9 Tabulation of individual Participant Data

None

10.4.10 Exploratory Analyses

The exploratory objectives are to:

- To assess efficacy of KK-LC-1 TCR T plus aldesleukin for the treatment of KK-LC-1+ cancers. The clinical response rates found at the MTD will be reported along with a 95% confidence interval and noted to be an exploratory result.
- To conduct exploratory immunologic studies to understand and improve the administered treatment. Any statistical tests performed on research samples for this purpose will be considered exploratory with results presented without formal adjustment but interpreted in the context of the number of tests performed.

11 COLLABORATIVE AGREEMENTS

11.1 COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA)

A CRADA between NCI and T-Cure Bioscience, Inc. is in place (CRADA #03313).

11.2 MULTI-INSTITUTIONAL GUIDELINES

Documents requiring submission to the reviewing IRB as provided in the Participating Site Information Sheet should be provided to the coordinating center for submission to the IRB.

12 HUMAN SUBJECTS PROTECTIONS

12.1 RATIONALE FOR SUBJECT SELECTION

The participants to be entered in this protocol have metastatic or recurrent/refractory locally advanced epithelial cancers that are refractory to standard therapy, and limited life expectances.

Subjects from both genders and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one group compared to another. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between participant safety considerations and limitations on the number

of individuals exposed to potentially toxic and/or ineffective treatments on the one hand and the need to explore gender and ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to gender or to ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.

12.2 PARTICIPATION OF CHILDREN

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

12.3 RISK/BENEFIT ASSESSMENT

12.3.1 Known Potential Risks

Over 400 participants have been treated in the Surgery Branch, NCI with TIL. Early toxicities related specifically to the infusion of the cells (those which are seen immediately following cell infusion and prior to aldesleukin administration) are generally mild and include fevers, chills, headache, and malaise. Toxicities which occur following administration of aldesleukin but are thought to be related to the cells include immune mediated events such as vitiligo, transient uveitis, hearing loss and vestibular dysfunction. The use of the non-myeloablative regimen prior to cell administration increases the toxicity of this treatment as profound myelosuppression occurs in all participants. In 93 participants treated with TIL using the non-myeloablative chemotherapy regimen with or without total body irradiation, there was one treatment related death (NMA + 200 cGy TBI) due to an unexpected but preexisting diverticular abscess. In the 101 participants treated in a subsequent randomized trial, 2 treatment-related deaths occurred – both due to the TBI component of the treatment regimen.

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

- Cyclophosphamide: Marrow suppression, nausea, mucositis, rash, hemorrhagic cystitis, myocardial damage, alopecia, infertility, nausea and vomiting, SIADH.
- Fludarabine: Myelosuppression, fever and chills, nausea and vomiting, malaise, fatigue, anorexia, weakness, neurologic toxicity, and interstitial pneumonitis. Serious opportunistic infections have occurred in CLL participants treated with fludarabine.
- Antimicrobials in general: Allergic reactions, renal impairment, nausea, vomiting, hepatic damage, marrow suppression, photosensitivity.
- High-dose aldesleukin administration: A listing of these side effects in 525 participants who received 1,039 treatment courses are listed in **Appendix C.**

12.3.1.1 Cell infusion

The risks of KK-LC-1 TCR-T cells are not known as the agent has not been studied in humans. Experience with diverse types of cell therapy including tumor-infiltrating lymphocytes, CAR-T

cells, and TCR-T cells, indicates that the risk of cell therapy may include cytokine release syndrome (where the T cell therapy causes the release of chemicals called cytokines that aggressively ramp up the immune system), autoimmunity (an immune reaction against normal tissues) and neurotoxicity (damage to the nervous system). Cytokine release syndrome may only cause mild symptoms such as fever, fatigue, headache, rash, joint stiffness, and muscle aches. It can also cause severe symptoms such as low blood pressure, high fever, uncontrolled systemic inflammatory response, hemophagocytic lymphohistiocytosis (a rare condition in which certain types of white blood cells build up in organs and destroy other blood cells), shock, vascular leakage (where fluids and proteins leak out of small blood vessels and into surrounding tissues), disseminated intravascular coagulation (a serious disorder where proteins that control blood clotting become overactive), and multi-organ system failure.

Gene-engineering of T cells may carry a risk of leukemia due to insertional mutagenesis (a mutation caused by insertion of new genetic material into a normal gene), but this event has not been reported in clinical experience with T cell products. Gene-engineering may also carry a risk of replication competent retrovirus (RCR) infection, which could be caused by the recombination of viral and cellular components during vector manufacturing, but this event has not been reported with T cell products. Severe autoimmune toxicity has occurred with some engineered T cell therapies. Immune-related toxicities are autoimmune conditions that could affect any organ in the body after product administration. The risk of severe autoimmunity with KK-LC-1 TCR T-cells is mitigated by preclinical studies that indicate that KK-LC-1 expression is restricted to tumors and germ cells. It is also mitigated by use of a TCR with targeting domains from a human TCR that has been through human thymic selection (which causes cells with strong reactivity to self-antigens to be eliminated). It is also mitigated by experience with prior safe treatment of a patient with 74.7 billion TIL cells, 67% of which naturally expressed the KK-LC-1 TCR.

12.3.1.2 Biopsy

The risks associated with biopsies are pain and bleeding at the biopsy site. In order to minimize pain, local anesthesia will be used. Rarely, there is a risk of infection at the sampling site. CT guidance may be used in obtaining biopsies. If so, there will also be a risk of exposure to radiation from up to 3 CT scans. This radiation exposure is not required for medical care and is for research purposes only. The amount of radiation received in this study is 0.43 rem which is below the guideline of 5 rem per year allowed for research subjects by the NIH Radiation Safety Committee.

12.3.1.3 Blood Sampling

Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting.

12.3.1.4 Leukapheresis

There may be some tingling in the face, mouth and fingers due to the medicine used to keep the blood from clotting during the procedure. The nurses may give a calcium-containing antacid to take away this tingling. Rarely, people may experience lightheadedness or dizziness. Rare complications of this procedure are lowered blood pressure, lightheadedness, dizziness, nausea, possible problems with the cell separator machine which would not allow the red cells and plasma to be returned and bleeding or bruising where the needles are put in arms.

12.3.1.5 X-ray examination

An x-ray examination exposes participants to a small amount of radiation, corresponding to one-fifth of the dose a person gets each year from natural sources, such as the sun and the ground. This small amount of radiation is not considered dangerous.

12.3.1.6 Cardiac Stress Test

A cardiac stress test may check for risks of a possible heart problem or diagnose an existing heart problem. During a cardiac stress test, participants may be asked to walk or jog on a treadmill. While doing this, their heart will be watched on a monitor. Problems can happen during or after the test. Blood pressure may decrease, and participants may feel dizzy, lightheaded, and weak. They may feel their heart throbbing or have extra heartbeats. Participants may have a chest pain or heart attack. Doctors are there before, during, and after the test to help.

12.3.1.7 Electrocardiogram (ECG)

An electrocardiogram or ECG is a test that records the electrical activity of the heart. It is used to measure the rate and regularity of heartbeats as well as the size and position of the heart chambers, and the presence of any damage to the heart. For this test, participants will be asked to lie down, and small patches that have an adhesive edge with a gel in the middle, called electrodes, will be placed on arms, legs, and chest. The areas where the electrodes are placed will be cleaned and, if needed, some hair may be shaved or clipped to allow for better attachment of the electrodes. The adhesive from the patches may irritate the skin.

12.3.1.8 Echocardiogram (ECHO)

An echocardiogram is an ultrasound to evaluate heart structure and function. This test is very safe and is performed using a probe with gel placed on the chest.

12.3.1.9 Pulmonary Function Tests (PFT)

PFTs are safe for most participants; however, some may experience dizziness, shortness of breath and fainting. In rare PFTs may lead to a collapsed lung. In participants with asthma, PFTs may precipitate an asthma attack.

12.3.1.10 6 Minute Walk Test (6MWT)

This is a low risk medical assessment that measures how far participants can walk in 6 minutes. Participants may experience chest pain or breathing trouble during the assessment and should tell the doctor.

12.3.1.11 Intravenous Catheter

The risks associated with placing some catheters include pain, bleeding, infection and collapsed lung. The long-term risks of the catheter include infection and clotting of veins. It may be necessary to remove the catheter.

12.3.1.12 X-ray examination

An x-ray examination exposes participants to a small amount of radiation, corresponding to one-fifth of the dose a person gets each year from natural sources, such as the sun and the ground. This small amount of radiation is not considered dangerous.

12.3.1.13 Imaging Scans

CT, PET, and/ or MRI scans may be used. MRI scans can be claustrophobic. CT and PET scans expose a participant to radiation; the amount depends on the number of body areas scanned. In addition, CT, PET, and MRI scans involve use of contrast (oral and/or IV). An IV line may need to be inserted for administration of the contrast agent and can cause pain at the site where the IV is placed. There is also a small risk of bruising or infection. If a contrast agent is given with the scan, there is a small risk of having a reaction to the contrast. In the small group of participants who have a reaction, the most common symptoms are nausea, pain in the vein where the contrast was given, headache, a metallic or bitter taste in the mouth, and a warm or flushing feeling that lasts from 1-3 minutes. Rarely, these symptoms may require treatment. In very rare cases, people have had more severe allergic reactions that result in skin rashes, shortness of breath, wheezing, or lowering of the blood pressure.

12.3.1.14 Gadolinium enhanced MRI

The risks of an IV catheter include bleeding, infection, or inflammation of the skin and vein with pain and swelling.

Mild symptoms from gadolinium infusion occur in fewer than 1% of those who receive it and usually go away quickly. Mild symptoms may include coldness in the arm during the injection, a metallic taste, headache, and nausea. In an extremely small number, fewer than one in 300,000 people, more severe symptoms have been reported including shortness of breath, wheezing, hives, and lowering of blood pressure. Participants should not receive gadolinium if they previously had an allergic reaction to it. They will be asked about such allergic reactions before gadolinium is given.

People with kidney disease are at risk for a serious reaction to gadolinium contrast called "nephrogenic systemic fibrosis (NSF)". This condition always involves the skin and can also involve the muscles, joints and internal organs. NSF has resulted in a very small number of deaths. A blood test of kidney function may be done within the month before an MRI scan with gadolinium contrast. Participants will not receive gadolinium for a research MRI scan if the kidney function is below the safe level.

Most of the gadolinium contrast leaves the body in the urine. However, the FDA has issued a safety alert that indicates small amounts of gadolinium may remain in the body for months to years. The effects of the retained gadolinium are not clear. At this time, retained gadolinium has not been linked to health risks in people whose kidneys work well. Some types of gadolinium contrast drugs are less likely to remain in the body than others. In this study, we will use the gadolinium contrast drugs that are less likely to remain in the body. We will also give participants additional information called a "Medication Guide." Upon request, we will give individual information about retained gadolinium we see on their studies.

12.3.1.15 Radiation Exposure

Screening:

This research study involves exposure to radiation from a chest x-ray, CT scan, PET scan, or CT-guided biopsy. The amount of radiation exposure participants may receive from these procedures is equal to approximately 3.1 rem. A rem is a unit of absorbed radiation.

The PET scan and CT scan in this study will expose participants to the roughly the same amount of radiation as 10 years' worth of background radiation. Most of the time, this amount of extra radiation is not harmful. However, scientists believe that being exposed to too much radiation can cause harmful side effects. This could include getting a new cancer. We estimate that this could happen in about 1 out of every 1000 people who get a very large amount of extra radiation.

Treatment:

This research study involves exposure to radiation from chest x-ray, CT and PET scans, or CT-guided biopsies. The amount of radiation exposure from these procedures is equal to approximately 8.1 rem. A rem is a unit of absorbed radiation.

The chest x-ray, CT scans, PET scans, or CT-guided biopsies in this study will expose participants to the roughly the same amount of radiation as 27 years' worth of background radiation. Being exposed to too much radiation can cause harmful side effects such as an increase in the risk of cancer. The risk depends on how much radiation participants are exposed to. Please be aware that about 40 out of 100 people (40%) will get cancer during their lifetime, and 20 out of 100 (20%) will die from cancer. The risk of getting cancer from the radiation exposure in this study is 0.8 out of 100 (0.8%) and of getting a fatal cancer is 0.4 out of 100 (0.4%).

Total:

Every day, people are exposed to low levels of radiation that come from the sun and the environment around them. The average person in the United States receives a radiation exposure of 0.3 rem per year from these sources. This type of radiation is called "background radiation." This study will expose participants to more radiation than they get from everyday background radiation. No one knows for sure whether exposure to these low amounts of radiation is harmful to the body.

The chest x-ray, PET and CT scans in this study may expose participants to roughly the same amount of radiation as 37.4 years' worth of background radiation. Being exposed to too much radiation can cause harmful side effects such as an increase in the risk of cancer. The risk depends on how much radiation they are exposed to. Please be aware that about 40 out of 100 people (40%) will get cancer during their lifetime, and 20 out of 100 (20%) will die from cancer. The risk of getting cancer from the radiation exposure in this study is 1.1 out of 100 (1.1%) and of getting a fatal cancer is 0.6 out of 100 (0.6%).

12.3.2 Known Potential Benefits

The experimental treatment has a chance to provide clinical benefit though it is not known if it will do so.

12.3.3 Assessment of Potential Risks and Benefits

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

only appropriate to carry out this experimental treatment in the context of life threatening metastatic cancer.

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

12.4 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided as a physical or electronic document to the participant for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

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 remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant. Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below, with a manual (non-electronic) signature on the electronic document. When required, witness signature will be obtained similarly as described for the investigator and participant as described below.

Manual (non-electronic) signature on electronic document:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,
- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location but is not required.

Both the investigator and the participant will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigators, funding agency, the Investigational New Drug (IND) or Investigational Device Exemption (IDE) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and as applicable, Food and Drug Administration (FDA).

13.2 QUALITY ASSURANCE AND QUALITY CONTROL

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Council for Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

13.3 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the

design and conduct of this trial. The study leadership in conjunction with the National Cancer Institute has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

13.4 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the/each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site(s) and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NCI CCR.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

14 PHARMACEUTICAL AND INVESTIGATIONAL DEVICE INFORMATION

Note: The package insert/prescribing information for each commercial supply used should supersede any information included below.

14.1 INTERLEUKIN-2

14.1.1 Other Names

(Aldesleukin, proleukin, recombinant human interleukin-2)

14.1.2 How Supplied

Aldesleukin (interleukin-2) will be provided by the NIH Clinical Pharmacy Department from commercial sources.

14.1.3 Formulation/Reconstitution

Aldesleukin, NSC #373364, is provided as single-use vials containing 22 million IU (~1.3mg) IL-2 as a sterile, white to off-white lyophilized cake plus 50mg mannitol and 0.18 mg sodium dodecyl sulfate, buffered with approximately 0.17 mg monobasic and 0.89 mg dibasic sodium phosphate to a pH of 7.5 (range 7.2 to 7.8). The vial is reconstituted with 1.2 mL of Sterile Water for Injection, USP, and the resultant concentration is 18 million IU/mL or 1.1 mg/mL. Diluent should be directed against the side of the vial to avoid excess foaming. Swirl contents gently until completely dissolved. Do not shake. Since vials contain no preservative, reconstituted solution should be used with 24 hours.

14.1.4 Storage

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

14.1.5 Dilution/Stability

Reconstituted aldesleukin should be further diluted with 50 mL of 5% Human Serum Albumin (HSA). The HSA should be added to the diluent prior to the addition of IL-2. Dilutions of the reconstituted solution over a 1000-fold range (i.e., 1 mg/mL to 1 mcg/mL) are acceptable in either glass bottles or polyvinyl chloride bags. Aldesleukin is chemically stable for 48 hours at refrigerated and room temperatures, 2 to 30C. As there are no formal stability studies of aldesleukin diluted with HSA, the expiration time will be limited to 4 hours, per pharmacy guidance.

14.1.6 Administration

The dosage will be calculated based on total body weight. The final dilution of aldesleukin will be infused over 15 minutes. Aldesleukin will be administered as an inpatient.

14.1.7 Toxicities

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

14.2 FLUDARABINE

14.2.1 Description

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

14.2.2 How Supplied

It will be purchased by the NIH Clinical Pharmacy Department from commercial sources. Fludarabine is supplied in a 50 mg vial as a fludarabine phosphate powder in the form of a white, lyophilized solid cake or as an intravenous solution 25 mg/mL, 2 mL vial.

14.2.3 Stability

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

14.2.4 Storage

Intact vials should be stored refrigerated (2 to 8°C).

14.2.5 Administration

Fludarabine is administered as an IV infusion in 100 mL 0.9% sodium chloride, USP or 5% dextrose in water, USP over 30 minutes. The doses will be based on body surface area (BSA). If participant is obese (BMI >35), drug dosage will be calculated using practical weight as described in **Appendix F**.

14.2.6 Toxicities

At doses of 25 mg/m^2/day for 5 days, the primary side effect is myelosuppression; however, thrombocytopenia is responsible for most cases of severe and life-threatening hematologic toxicity. Serious opportunistic infections have occurred in CLL participants treated with fludarabine. Hemolytic anemia has been reported after 1 or more courses of fludarabine with or without a prior history of a positive Coomb's test; fatal hemolytic anemia has been reported. In addition, bone marrow fibrosis has been observed after fludarabine therapy. Other common adverse effects and potentially fatal central nervous system toxicity in the form of progressive encephalopathy, blindness, and coma is only rarely observed at the currently administered doses of fludarabine. More common neurologic side effects at the current doses of fludarabine include weakness, pain, malaise, fatigue, paresthesia, visual or hearing disturbances, and sleep disorders. Adverse respiratory effects of fludarabine include, cough, dyspnea, allergic or idiopathic interstitial pneumonitis. Tumor lysis syndrome has been rarely observed in fludarabine treatment of CLL. Treatment on previous adoptive cell therapy protocols in the Surgery Branch

have caused persistently low (below 200) CD4 counts, and 1 participant developed polyneuropathy manifested by vision blindness, and motor and sensory defects.

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

14.3 CYCLOPHOSPHAMIDE

14.3.1 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.

14.3.2 How Supplied

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

14.3.3 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 8C.

14.3.4 Administration

It will be diluted in 100 to 250 mL of 0.9% sodium chloride, USP or 5% dextrose in water, USP and infused over approximately 30-60 minutes. The dose will be based on the participant's body weight. If participant is obese (BMI>35) drug dosage will be calculated using practical weight as described in **Appendix F**.

14.3.5 Toxicities

Hematologic toxicity occurring with cyclophosphamide usually includes leukopenia and thrombocytopenia. Anorexia, nausea and vomiting, rash and alopecia occur, especially after high-dose cyclophosphamide; diarrhea, hemorrhagic colitis, infertility, and mucosal and oral ulceration have been reported. Sterile hemorrhagic cystitis occurs in about 20% of participants; severity can range from microscopic hematuria to extensive cystitis with bladder fibrosis. Although the incidence of hemorrhagic cystitis associated with cyclophosphamide appears to be lower than that associated with ifosfamide, mesna (sodium 2-mercaptoethanesulfonate) has been used prophylactically as an uroprotective agent in participants receiving cyclophosphamide. Prophylactic mesna is not effective in preventing hemorrhagic cystitis in all participants. Participants who receive high dose cyclophosphamide may develop interstitial pulmonary fibrosis, which can be fatal. Hyperuricemia due to rapid cellular destruction may occur, particularly in participants with hematologic malignancy. Hyperuricemia may be minimized by adequate hydration, alkalinization of the urine, and/or administration of allopurinol. If allopurinol is administered, participants should be watched closely for cyclophosphamide toxicity (due to allopurinol induction of hepatic microsomal enzymes). At high doses, cyclophosphamide can result in a syndrome of inappropriate antidiuretic hormone secretion; hyponatremia with progressive weight gain without edema occurs. At high doses,

cyclophosphamide can result in cardiotoxicity. Deaths have occurred from diffuse hemorrhagic myocardial necrosis and from a syndrome of acute myopericarditis; in such cases, congestive heart failure may occur within a few days of the first dose. Other consequences of cyclophosphamide cardiotoxicity include arrhythmias, potentially irreversible cardiomyopathy, and pericarditis. Other reported adverse effects of cyclophosphamide include headache, dizziness, and myxedema; faintness, facial flushing, and diaphoresis have occurred following IV administration. Mesna (sodium 2-mercaptoethanesulphonate; given by IV injection) is a synthetic sulfhydryl compound that can chemically interact with urotoxic metabolites of cyclophosphamide (acrolein and 4-hydroxycyclophosphamide) to decrease the incidence and severity of hemorrhagic cystitis.

14.4 CELL PREPARATION (KK-LC-1 TCR TRANSDUCED PBL) (IND # 27348)

The procedure for the expanding the human PBL and the Certificate of Analysis (CoA) are similar to those approved by the Food and Drug Administration and used at the NCI in ongoing protocols. The CoA is included in **Appendix E**. The PBL will be transduced with retroviral supernatant containing the KK-LC-1 TCR.

14.4.1 Retroviral Vector Containing the KK-LC-1 TCR gene

The retroviral vector supernatant (PG13-MSGV1-KK-LC-1-TCR) encoding a T cell receptor directed against KK-LC-1 (KK-LC-1_{52-60}) was prepared and preserved following cGMP conditions in the Cincinnati Children's Hospital Vector Production Facility (CCHMC). The KK-LC-1 TCR vector was produced by the Cincinnati Children's Hospital Vector Production Facility. The backbone is the MSGV1 retrovirus that has been used in prior gene therapy clinical trials. It was produced using a PG13-based packaging line.

The retroviral vector KK-LC-1 TCR consists of 7,313 bps including the 5'LTR from the murine stem cell virus (promoter), packaging signal including the splicing donor (SD) and splicing acceptor sites, alpha and beta chain genes of the KK-LC-1 TCR. The alpha and beta chains are linked by a P2A peptide. The vector was codon optimized for expression by human cells with constant region exchanged for murine counterparts with an added disulfide bond and hydrophobic substitutions in the alpha chain constant region transmembrane domain.

The physical titer will be determined by transduction of PBL with serial dilutions of the vector. TCR expression on the cell surface will be measured using FACS following staining with an anti-mouse constant region antibody. The titer will be measured as transducing units per milliliter. Portions of the supernatant will be stored at ≤ -65C at CCHMC (for long term stability testing), NCI at Frederick Central Repository and the NIH Clinical Center, Center for Cellular Engineering. These storage facilities are equipped with around-the-clock temperature monitoring. Upon request, supernatant will be delivered on dry ice to be used in *ex vivo* transduction of participant PBL. There will be no re-use of the same unit of supernatant for different participants. 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://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_sect_IV.pdf.

14.5 MESNA

14.5.1 Other Names

Sodium 2-mercaptoethanesulfonate, Mesnum, Mesnex, NSC-113891

14.5.2 How Supplied

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

14.5.3 Storage

Intact ampules are stored at room temperature.

14.5.4 Stability

Diluted solutions (1 to 20 mg/mL) are physically and chemically stable for at least 24 hours under refrigeration. Mesna is chemically stable at room temperature for 48 to 72 hours in D5W, 48 to 72 hours in D5W/0.45% NaCl, or 24 hours in 09% NaCl.

14.5.5 Administration

Dilute to concentrations less than or equal to 20 mg Mesna/mL fluid in D5W or 0.9% NaCl and to be administered intravenously as a continuous infusion. If participant is obese (BMI >35) drug dosage will be calculated using practical weight as described in table 6 (FDA-approved package insert).

14.5.6 Toxicities

Include nausea, vomiting, and diarrhea.

14.6 FILGRASTIM

Note: Filgrastim or its biosimilar may be used interchangeably on this protocol.

14.6.1 Other Names

Granulocyte Colony-Stimulating Factor, G-CSF, Filgrastim, Neupogen

14.6.2 How supplied

Filgrastim will be obtained commercially by the Clinical Center Pharmacy Department and is supplied in 300 ug/mL and 480 ug/1.6mL vials.

14.6.3 Storage

G-CSF should be refrigerated and not allowed to freeze. The product bears the expiration date. The product should not be shaken.

14.6.4 Stability

It is generally stable for at least 10 months when refrigerated.

14.6.5 Administration

The appropriate dose is drawn up into a syringe. G-CSF will be given as a daily subcutaneous injection.

14.6.6 Toxicities

The side effects of G-CSF are skin rash, myalgia, and bone pain, an increase of pre-existing inflammatory conditions, enlarged spleen with occasional associated low platelet counts, alopecia (with prolonged use) elevated blood chemistry levels.

14.7 TRIMETHOPRIM AND SULFAMETHOXAZOLE DOUBLE STRENGTH (TMP/SMX DS)

14.7.1 How supplied

TMP/SMX DS will be obtained by the Clinical Center Pharmacy Department from commercial sources.

It may be used for the prevention of PCP pneumonia.

14.7.2 Administration

The oral dose is 1 tablet PO daily 3 times a week (MUST be on non-consecutive days) beginning at or near the time of discharge from the hospital and continuing for at least 6 months and until the CD4 count is greater than 200 on 2 consecutive lab studies.

14.7.3 Toxicities

Like other sulfa drugs, TMP/SMX DS can cause allergies, fever, photosensitivity, nausea, and vomiting. Allergies typically develop as a widespread itchy red rash with fever 8 to 14 days after beginning the standard dose. Neutropenia, a reduction in the number of neutrophils, can also occur.

14.8 AEROSOLIZED PENTAMIDINE IN PLACE OF TMP/SMX DS

Participants with sulfa allergies may receive aerosolized Pentamidine 300 mg per nebulizer at or around the time of discharge from the hospital and continued monthly until the CD4 count is above 200 on 2 consecutive follow up lab studies and for at least 6 months post chemotherapy. Pentamidine Isethionate will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prevent the occurrence of PCP infections. It is supplied in 300 mg vials of lyophilized powder and will be administered via nebulizer. Toxicities reported with the use of Pentamidine include metallic taste, coughing, bronchospasm in heavy smokers and asthmatics; increased incidence of spontaneous pneumothorax in participants with previous PCP infection or pneumatoceles, or hypoglycemia.

14.9 HERPES VIRUS PROPHYLAXIS

14.9.1 Valacyclovir (Valtrex)

Valacyclovir will be obtained by the Clinical Center Pharmacy Department from commercial sources. It may be used orally to prevent the occurrence of herpes virus infections in participants with positive HSV serology. It is supplied in 500 mg tablets. Valacyclovir may be started on day 0 at a dose of 500 mg orally daily if the participant is able to tolerate oral intake. See package insert for dosing adjustments in participants with renal impairment. Common side effects include headache, upset stomach, nausea, vomiting, diarrhea, or constipation. Rare serious side effects include hemolytic uremic syndrome and thrombotic thrombocytopenic purpura.

14.9.2 Acyclovir

Acyclovir will be obtained by the Clinical Center Pharmacy Department from commercial sources. It may be used to prevent the occurrence of herpes virus infections in participants who cannot take oral medications. It is supplied as powder for injection in 500 mg/vials. Reconstitute in 10 mL of sterile water for injection to a concentration of 50 mg/mL. Reconstituted solutions should be used within 12 hours. IV solutions should be diluted to a

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

14.10 Fungal Prophylaxis

14.10.1 Fluconazole

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

14.11 Support Medications

14.11.1 Ondansetron hydrochloride

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

14.11.2 Furosemide

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

14.12 KK-LC-1 Positivity Assay (NSR Device)

In order to be eligible for the study, participants are required to have histologically or cytologically confirmed KK-LC-1 positive epithelial cancer. KK-LC-1 positivity assay is not FDA approved for this purpose; however, it is being used as a diagnostic device. Validation assays to support the use of the assay have been submitted to IND. All of the documentation is in the IND files.

According to 21 CFR 812.3(m), a significant risk device presents a potential for serious risk to the health, safety and welfare of a subject and meets the significant risk criteria listed in the table below along with the sponsor's conclusions with regard to the applicability of these criteria to the current study. The device has been assessed by the sponsor as non-significant risk per the below.

	Applicable to current study	**Justification**
Is an implant	No	The KK-LC-1 positivity assay is not introduced into the subject
Is used in supporting or sustaining human life	No	The device is diagnostic
Is of substantial importance in diagnosing mitigating or treating disease or preventing impairment of human health	No	While the device is diagnostic, we do not believe it presents a potential for serious risk to the health and welfare of the subject. The assessment of KK-LC-1 positivity is only used to determine eligibility for the study and is assessed to help to increase the possibility that all persons enrolling on the study might derive benefit from therapy. Persons that are deemed ineligible to enroll on the basis of this test are eligible for studies within CIO that are not reliant on this test.
Otherwise poses a risk	No	Testing will be performed on archival samples or on fresh tissue that is collected at screening for confirmation of diagnosis. No additional collection of tissue will occur for purposes of KK-LC-1 testing.

Source: Testing will be performed via an IHC assay developed by Rutgers Cancer Institute of New Jersey.

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16 APPENDICES

16.1 APPENDIX A: ADVERSE EVENTS OCCURRING IN ≥10% OF PARTICIPANTS TREATED WITH ALDESLEUKIN (N=525)[1]

Body System	% Participants	Body System	% Participants
Body as a Whole		*Metabolic and Nutritional Disorders*	
Chills	52	Bilirubinemia	40
Fever	29	Creatinine increase	33
Malaise	27	Peripheral edema	28
Asthenia	23	SGOT increase	23
Infection	13	Weight gain	16
Pain	12	Edema	15
Abdominal pain	11	Acidosis	12
Abdomen enlarged	10	Hypomagnesemia	12
Cardiovascular		Hypocalcemia	11
Hypotension	71	Alkaline phosphatase incr	10
Tachycardia	23	*Nervous*	
Vasodilation	13	Confusion	34
Supraventricular tachycardia	12	Somnolence	22
Cardiovascular disorder[a]	11	Anxiety	12
Arrhythmia	10	Dizziness	11
Digestive		*Respiratory*	
Diarrhea	67	Dyspnea	43
Vomiting	50	Lung disorder[b]	24
Nausea	35	Respiratory disorder[c]	11
Stomatitis	22	Cough increase	11
Anorexia	20	Rhinitis	10
Nausea and vomiting	19	*Skin and Appendages*	
Hemic and Lymphatic		Rash	42
Thrombocytopenia	37	Pruritus	24
Anemia	29	Exfoliative dermatitis	18
Leukopenia	16	*Urogenital*	
		Oliguria	63

a Cardiovascular disorder: fluctuations in blood pressure, asymptomatic ECG changes, CHF.

b Lung disorder: physical findings associated with pulmonary congestion, rales, rhonchi.

c Respiratory disorder: ARDS, CXR infiltrates, unspecified pulmonary changes.

[1]Source: Proleukin® Prescribing Information – June 2007

16.2 APPENDIX B: EXPECTED IL-2 TOXICITIES AND THEIR MANAGEMENT

Toxicity	Grade	Supportive Medications	Stop Cycle*	Stop Treatment**
Chills	3	IV Meperidine 25-50mg IV q1hr, prn	No	No
Fever	3	Acetaminophen 650mg po q4hr; Indomethacin 50-75mg po q8h	No	No
Pruritus	3	Hydroxyzine HCl 10-20mg po q6h, prn; Diphenhydramine HCl 25-50mg po q4h prn	No	No
Nausea/Vomiting/Anorexia	3	Ondansetron 10mg IV q8hr prn, Granisetron 0.01 mg/kg IV qday prn, Droperidol 1mg IV a4-6h prn; Prochlorperazine 25mg PR prn or 10mg IV q6hr prn	No	No
Diarrhea	3	Loperamide 2mg po q3h prn; Diphenoxylate HCl 2.5mg and Atropine sulfate 25mcg po q3h prn; Codeine sulfate 30-60mg po q4h prn	If uncontrolled after 24h despite all supportive measures	No
Malaise	3 or 4	Bedrest	If other toxicities occur simultaneously	No
Hyperbilirubinemia	3 or 4	Observation	If other toxicities occur simultaneously	No
Anemia	3 or 4	Transfusion with PRBCs	If uncontrolled despite all supportive measures	No
Thrombocytopenia	3 or 4	Transfusion with platelets	If uncontrolled despite all supportive measures	No

Toxicity	Grade	Supportive Medications	Stop Cycle*	Stop Treatment**
Edema/Weight gain	3	Diuretics prn	No	No
Hypotension	3	Fluid resuscitation, Vasopressor support	If uncontrolled despite all supportive measures	No
Dyspnea	3 or 4	Oxygen or ventilator support	If requires ventilator support	No
Oliguria	3 or 4	Fluid boluses or dopamine at renal doses	If uncontrolled despite all supportive measures	No
Increased Creatinine	3 or 4	Observation	Yes (Grade 4)	No
Renal Failure	3 or 4	Dialysis/CVVH	Yes	Yes
Pleural Effusion	3	Thoracentesis	If uncontrolled despite all supportive measures	No
Bowel Perforation	3	Surgical intervention	Yes	Yes
Confusion	3	Observation	Yes	No
Somnolence	3 or 4	Intubation for airway protection	Yes	Yes
Arrhythmia	3	Correction of fluid and electrolyte imbalances; chemical conversion or electrical conversion therapy	If uncontrolled despite all supportive measures	No
Elevated Troponin Levels	3 or 4	Observation	Yes	If changes in LV function have not improved to baseline by next dose

Toxicity	Grade	Supportive Medications	Stop Cycle*	Stop Treatment**
Myocardial Infarction	4	Supportive care	Yes	Yes
Elevated Transaminases	3 or 4	Observation	For Grade 4 without liver metastases	If changes have not improved to baseline by next dose
Hyperbilirubinemia	3 or 4	Observation	For Grade 4 without liver metastases	If changes have not improved to baseline by next dose
Electrolyte Imbalances	3 or 4	Electrolyte replacement	If uncontrolled despite all supportive measures	No
Neutropenia	4	Observation	No	No

*Unless the toxicity is not reversed within 12 hours.

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

16.3 APPENDIX C: INTERLEUKIN-2 TOXICITIES OBSERVED IN PARTICIPANTS TREATED AT THE NIH CLINICAL CENTER

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

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

* Eleven patients are in two protocols.

16.4 APPENDIX D: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale	
Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

16.5 APPENDIX E: CERTIFICATE OF ANALYSIS KK-LC-1 TCR

Date of preparation of final product:

Participant:

Tests performed on final product: *Test*	*Method*	*Limits*	*Results*	*Initials/Date*
Cell viability[1]	Trypan blue exclusion	>70%		
Total viable cell number[1]	Visual microscopic count	$>1x10^9$		
TCR expression[2]	FACS analysis of the transduced cells	PBL, >10%		
Microbiological studies	Gram stain[1,3]	No micro-organisms seen		
	Aerobic culture[3,4]	No growth		
	Fungal culture[3,4]	No growth		
	Anaerobic culture[3,4]	No growth		
	Mycoplasma test[5]	Negative		
Endotoxin	Limulus assay[1]	<5 E.U./kg		
RCR	RCR-PCR[6]	Negative		

[1]Performed on sample of the final product immediately prior to infusion. Results are available at the time of infusion.

[2]Performed 2 to 10 days post-transduction. Results are available at the time of infusion.

[3]Performed 2 to 4 days prior to infusion. Results are available at the time of infusion but may not be definitive.

[4]Sample collected from the final product prior to infusion. Results will not be available before cells are infused into the participant.

[5]Performed 2 to 10 days prior to infusion. Results are available at the time of infusion.

[6]Performed on sample approximately 1 to 4 days prior to infusion. Results are available at the time of infusion.

Prepared by: _____ Date:_____

QC sign-off: _____ Date: _____

Qualified Clinical or Laboratory Supervisor

16.6 APPENDIX F: MODIFICATION OF DOSE CALCULATIONS* IN PARTICIPANTS WHOSE BMI IS GREATER THAN 35

1. **BMI Determination:**

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

2. **Calculation of ideal body weight**

   Male = 50kg + 2.3 (number of inches over 60 inches)

   Example: ideal body weight of 5'10" male
   50 + 2.3 (10) = 73 kg

   Female = 45.5kg + 2.3 (number of inches over 60 inches)

   Example: ideal body weight of a 5'3" female
   45.5 + 2.3 (3) = 57kg

3. **Calculation of "practical weight"**

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

16.7 APPENDIX G: INFUSION REACTIONS – GUIDELINES FOR MANAGEMENT

NCI-CTCAE 5.0 Grade	Treatment Modification for Infused Agent
Grade 1 – mild Mild transient reaction; infusion interruption not indicated; intervention not indicated.	Consider decreasing the infusion rate of the particular agent by 50% and monitoring closely for any worsening.
Grade 2 – moderate Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDs, narcotics, i.v. fluids); prophylactic medications indicated for ≤ 24 hours.	Consider temporarily discontinuing infusion of the particular agent. Consider resuming infusion of the particular agent at 50% of previous rate once infusion related reaction has resolved or decreased to at least Grade 1 in severity and monitor closely for any worsening.
Grade 3 or Grade 4 – severe or life-threatening Grade 3: Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); hospitalization indicated for clinical sequelae. Grade 4: Life-threatening consequences; urgent intervention indicated.	Stop the infusion immediately and disconnect infusion tubing from the subject. For grade 3 events: Consider withdrawing immediately from treatment with that particular agent and not offering any further treatment with that agent based upon if the clinical condition can be safely managed. For grade 4 events: Withdraw immediately from treatment and do not offer further treatment with that agent.

16.8 APPENDIX H: PARTICIPATING SITE ROLES AND OVERSIGHT PLAN

The study will also be conducted at a participating site under a reliance agreement. The role of the participating site is limited to sample and data analysis as described in sections **5.1.5**. Enrollment and routine follow up per protocol will not be conducted at participating site.

Data will be collected from the sites as outlined in section **6.1** and expedited reporting will be per section **7.4**. Protocol and regulatory compliance will be ensured as described in section **7.6.1** and **13.2**.

IRB determinations will be forwarded to the participating site PI/study coordinator by a member of the study team.