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PROTOCOL TITLE

A Phase I/II Study Administering Peripheral Blood Lymphocytes Transduced with a Murine T-Cell Receptor Recognizing the G12D Variant of Mutated RAS in HLA-A*11:01 Patients

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Drug Name:	Anti-KRAS G12D murine TCR transduced PBL	Cyclophosphamide	Fludarabine	Aldesleukin
IND Number:	18470	18470	18470	18470
Sponsor:	Center for Cancer Research	Center for Cancer Research	Center for Cancer Research	Center for Cancer Research
Manufacturer:	Surgery Branch Cell Production Facility	Generic	Generic	Generic
Supplier:	Surgery Branch Cell Production Facility	CC Pharmacy	CC Pharmacy	CC Pharmacy

PRÉCIS

Background:

- We generated an HLA-A*11:01-restricted murine T-cell receptor (mTCR) that specifically recognizes the G12D-mutated variant of KRAS (and other RAS family genes), expressed by many human cancers and constructed a single retroviral vector that contains alpha and beta chains that confer recognition of this antigen when transduced into PBL.
- In co-cultures with HLA-A*11:01+ target cells expressing this mutated oncogene, mTCR transduced T-cells lyse target cells and secrete IFN-gamma with high specificity.

Objectives:

- Primary objectives:
  - Phase I: Determine the safety of administering PBL transduced with anti-KRAS G12D mTCR in concert with preparative lymphodepletion and high-dose interleukin-2 (IL-2; aldesleukin).
  - Phase II: Determine if anti-KRAS G12D mTCR-transduced PBL can mediate the regression of tumors harboring the RAS G12D mutation.

Eligibility:

- Patients must be/have:
  - Age ≥ 18 years and ≤ 72 years
  - HLA-A*11:01 positive
  - Metastatic or unresectable RAS G12D-expressing cancer which has progressed after standard therapy (if available).
- Patients may not have:
  - Allergies or hypersensitivities to high-dose aldesleukin, cyclophosphamide, or fludarabine.

Design:

- This is a phase I/II, single center study of PBL transduced with anti-KRAS G12D mTCR in HLA-A*11:01 positive patients with advanced solid tumors expressing G12D mutated RAS.
- PBMC obtained by leukapheresis 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 these cells to retroviral vector supernatant containing replication-incompetent virus encoding the anti-KRAS G12D mTCR.
- All patients will receive a non-myeloablative, lymphodepleting preparative regimen of cyclophosphamide and fludarabine.
- On Day 0, patients will receive their PBL transduced with the anti-KRAS G12D mTCR and will then begin high-dose aldesleukin.

- A complete evaluation of lesions will be conducted approximately 6 weeks (± 2 weeks) after treatment.
- The study will be conducted using a phase I/II Simon minimax design, with two separate cohorts for the Phase II component: Cohort 2a, patients with RAS G12D pancreatic cancer, and Cohort 2b, patients with RAS G12D non-pancreatic cancer.
- A total of up to 70 patients may be required; approximately 24 patients in the Phase I portion of the study and 46 (21, plus an allowance of up to 2 non-evaluable per Phase II cohort) patients in the Phase II portion of the study.

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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 Objectives

- Phase I: Determine the safety of administering PBL transduced with anti-KRAS G12D mTCR in concert with preparative lymphodepletion and high-dose interleukin-2 (IL-2; aldesleukin).
- Phase II: Determine if anti-KRAS G12D mTCR-transduced PBL can mediate the regression of tumors harboring the RAS G12D mutation.

1.1.2 Exploratory Objective

- Determine the *in vivo* survival of mTCR gene-engineered cells.

1.2 BACKGROUND AND RATIONALE

1.2.1 Adoptive Cell Transfer Experience at the NCI Surgery Branch

The National Cancer Institute Surgery Branch (NCI-SB) has pioneered novel T-cell based cancer therapies for chemotherapy-refractory cancers and continues expansion of these efforts. This work has its foundation in the successful treatment of metastatic cutaneous melanoma with adoptive transfer of tumor-infiltrating lymphocytes (TIL). We have reported the results of adoptive transfer therapy in 93 patients with metastatic melanoma who received TIL following a lymphodepleting regimen plus aldesleukin administration, with or without total body irradiation (TBI)[1]. Forty-three patients received a non-myeloablative, lymphodepleting preparative regimen consisting of 60 mg/kg cyclophosphamide daily for two days and 25 mg/m^2 fludarabine daily for five days prior to cell transfer and aldesleukin administration. Twenty-five patients each also received the same chemotherapy agents in conjunction with either 200 or 1200 cGy TBI prior to cell infusion and aldesleukin administration. The overall objective response rate using RECIST criteria in these 93 patients was 56%, with 20 of the 93 patients achieving complete responses

and only one of the 20 patients ever relapsing. The response rates and overall survival in this trial are shown in **Figure 1** and **Table 1**. There was one treatment-related death among these 93 patients, which occurred in a patient who received 200 cGy TBI and who had an undetected diverticular abscess prior to beginning therapy. Of the 52 responding patients in this trial, 42 had disease that was refractory to aldesleukin therapy and 22 had disease that was refractory to prior aldesleukin plus chemotherapy. A follow-up study randomizing 101 patients with metastatic melanoma to either cyclophosphamide and fludarabine alone, or cyclophosphamide and fludarabine plus 1200 cGy TBI, was also completed (no difference has been demonstrated between the two arms, so the 101 patients are considered together). In that study, an overall response rate of 53% was observed with 24% of patients achieving complete responses. Only one of these 101 patients with complete responses has relapsed. These TIL studies in patients with metastatic melanoma demonstrate that adoptive T-cell therapy can be a highly effective treatment even when other therapies have failed.

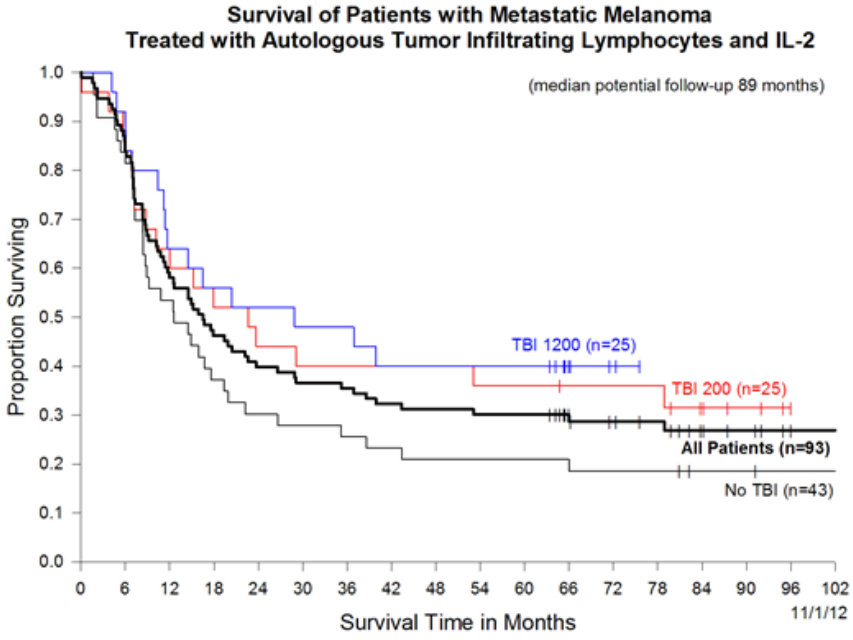


Figure 1. *Survival of patients with metastatic melanoma treated with autologous TIL and IL-2 following three different lymphoconditioning regimens. Nineteen of these 93 patients are in maintained complete responses beyond 5 years.*

Cell Transfer Therapy (4/1/15)

Treatment	Total	PR	CR	OR (%)
		number of patients (duration in months)		
No TBI	43	16 (37%) (84, 36, 29, 28, 14, 12, 11, 7, 7, 7, 7, 4, 4, 2, 2, 2)	5 (12%) (137+,135+,134+, 120+, 82+)	21 (49%)
200 TBI	25	8 (32%) (14, 9, 6, 6, 5, 4, 3, 3)	5 (20%) (124+,120+,116+, 113+, 64+)	13 (52%)
1200 TBI	25	8 (32%) (21, 13, 6, 6, 6, 5, 3, 2)	10 (40%) (104+,101+,100+, 95+, 94+, 94+, 94+, 93+, 63+, 19)	18(72%)

(20 complete responses: 19 ongoing at 63 to 137 months)

Table 1. *Response rates and duration in patients with melanoma treated with TIL plus high-dose aldesleukin following three different lymphoconditioning regimens.*

1.2.2 Gene Engineering PBL with Specific Antigen-Reactive T-Cell Receptors

TIL have been a consistent source of tumor-reactive T-cells in patients with melanoma, but it has been difficult to obtain TIL with autologous tumor recognition from other tumor types. T-cells recognize short peptide fragments (8-14 amino acids) that are presented on the surface of class I or II major histocompatibility (MHC) molecules. The molecule that recognizes these peptide fragments in the context of MHC is the alpha-beta heterodimeric T-cell receptor (TCR). Beginning in 2006, several clinical studies reported that peripheral blood lymphocytes (PBL) can be retrovirally engineered with specific TCRs to recognize a tumor-associated antigen and can cause tumor regression when re-administered to patients[2, 3]. Initially, these introduced TCRs were reactive with proteins involved in melanogenesis and were used in patients with metastatic melanoma where tumor regressions were seen. Because the target antigens were present on both melanoma and normal melanocytes, patients developed vitiligo, uveitis, and reduced hearing due to the presence of normal melanocytes in these anatomic sites[4]. A similar scenario occurred when T-cells redirected to recognize carcinoembryonic antigen (CEA) were used in patients with colon cancer, with unacceptable colitis developing due to expression of CEA in some normal gastrointestinal epithelial cells[5]. Although tumor regressions were seen, it was thought that such normal 'self-antigens' expressed as differentiation antigens in normal organs as well as by cancers arising from these organs, would ultimately not be useful. Subsequently, the concept of using receptor re-engineered T-cells to treat cancer has been validated by using TCRs directed against other classes of antigens such as the tumor-germline family of proteins. One of these, NY-ESO-1, was targeted by autologous PBL transduced with an HLA-A2 restricted TCR[6]. A phase II clinical trial was conducted targeting NY-ESO on melanomas and synovial sarcomas with TCR-engineered PBL in combination with preparative lymphodepleting chemotherapy and systemic IL-2. A total of 20 patients with metastatic melanoma and 18 patients with synovial

sarcoma (which frequently contains a chromosomal translocation that results in high NY-ESO expression) were enrolled (**Table 2**). In total, objective partial or complete responses were seen in 55% (11/20) of patients with melanoma, four of which are ongoing, and in 61% (11/18) of patients with synovial sarcoma, two of which are ongoing. These results demonstrate the efficacy of TCR-engineered cells in adoptive therapy when normal tissue toxicity could be avoided.

Table 2. *Response to therapy with NY-ESO-1 TCR (02/01/2015)*

	Total	PR (%)	CR (%)	OR (%)
Melanoma	**20***	**7 (35%)** α (28, 10, 8, 5+, 5, 3, 3)	**4 (20%)** (58+, 54+, 40+, 24)	**11 (55%)**
Synovial Cell Sarcoma	**18**	**10 (55%)** (47+, 11, 10, 8, 7, 6, 5, 4, 3, 3)	**1 (6%)** 24+	**11 (61%)**

α Durations of response in months are in parenthesis under the number of responders.

"+" indicates an ongoing response.

* 2 of 22 patients were not evaluable for response.

Another clinical trial targeting the CD19 determinant on B-cell lymphomas with a chimeric antigen receptor (CAR) has demonstrated that anti-tumor efficacy can be achieved when the accompanying normal tissue toxicity is tolerable. In that trial, the introduced receptor was a CAR consisting of an immunoglobulin-derived extracellular binding moiety and the intracellular signaling molecule zeta of the CD3 complex on T-cells. When PBL were engineered with this receptor and attacked CD19-bearing target cells, high rates of regression in multiple treatment-refractory B-cell malignancies were seen[7-9]. Due to targeting CD19 antigen, normal B-cells were also completely deleted. With IgG support and infection prophylaxis, this normal tissue toxicity has proven to be manageable, allowing this approach to be used to good effect against multiple types of B-cell tumors. Thus, targeting some antigens expressed by both tumors and normal tissues can be safe and effective if the toxicity can be tolerated or managed.

Genetically redirected T-cells have the potential for expected and unexpected toxicities. When normal self-antigens are the target, there can be predictable autoimmunity. Our experiences with the melanocyte/melanoma antigens caused vitiligo and uveitis and severe colitis was seen when CEA was targeted. This illuminates a major problem with targeting normal self-proteins. Off-target toxicities can be harder to predict. In a study at the University of Pennsylvania using TCR-transduced cells that recognized the HLA-A1 class I restricted epitope from MAGE-A3 (aa 168-176), cardiac toxicity was attributed to changes introduced into the binding regions of the[10] TCR, which added new specificities resulting in cardiac rejection. Four amino acid changes were made in the CDR2 region of the alpha chain of the TCR which led to the recognition of the titin protein and thought to be the cause of the off-target toxicity. Unmodified TCR did not recognize titin and these investigators could find no MAGE-A3 expression in the heart. One NCI-SB protocol using a mTCR also resulted in off-target toxicities. In that protocol targeting MAGE-A3, the

introduced TCR recognized a highly homologous MAGE-family member, MAGE-A12, expressed in areas of the brain causing irreversible CNS damage in two patients.

1.2.3 T-Cells Recognizing "Neoantigens" Generated by Tumor-Specific Mutations

Recently, evidence has accumulated that much of the endogenous anti-tumor T-cell response is directed against "neoantigens" generated by tumor-specific somatic mutations. Although sporadic neoantigen recognition was encountered during efforts to identify the antigens recognized by TIL from melanoma,[11] this was first studied in detail by Robbins in 2013[12]. Whole exome sequencing of melanomas revealed high rates of mutation, likely due to UV mutagenesis. Algorithms predicting the binding of all putative mutated tumor-associated epitopes to the patient's MHC alleles were used to rank predicted epitopes by MHC affinity. Then the 25-40 highest predicted binders were synthesized and the TIL screened for recognition of those peptides. Melanoma TIL recognized one or more of the predicted epitopes in this short list of candidates. Since then, recognition of mutated epitopes has been found in additional melanoma patients and patients with a wide variety of gastrointestinal adenocarcinomas[13]. One patient with cholangiocarcinoma even received a near clonal population of mutation reactive T-cells and has a major tumor regression still ongoing at two years[14]. Other investigators have shown that the tumor histologies most responsive to checkpoint inhibitors blocking CTLA-4 and PD-1 are often those with high rates of mutations, and higher individual mutation rates are associated with a greater chance of responding to these immunotherapies[15, 16]. Perhaps most persuasive is that the response rates of colon cancer to anti-PD-1 antibodies is higher in patients with cancers with mismatch repair defects and in lung cancer, to smokers compared to non-smokers—in both cases, response rates are higher in the subpopulation with higher rates of mutation[17]. Such neoantigens are perhaps the optimal tumor-rejection antigens since they are totally tumor-specific and the immune repertoire against them has not been edited by the thymus, which eliminates high avidity reactivity against "self". The main drawbacks to targeting these antigens are the need to do whole exome sequencing, the potential need to devise a different treatment for every patient, and the possibility that heterogeneity in the mutations of cancer cells within a patient would rapidly lead to resistance. One way to address all of these problems is if high-frequency, ubiquitous mutations (which are likely to be driver mutations present in nearly all cells of a patient's cancer) can be found that are immunogenic. Unfortunately, the most prominent driver mutations in human cancers are often mutated in hundreds of different ways, making it difficult to target them immunologically.

1.2.4 Targeting Mutated RAS

Mutations in the KRAS gene are one of the most common genetic abnormalities in human cancer. Parallel mutations in the RAS family genes NRAS and HRAS also occur in human tumors. It is estimated that the KRAS pathway is abnormal in nearly a third of human cancers. These mutated RAS family oncogenes are the prototypical driver mutations discovered in human cancers and appear early on in tumor development. The fact that they occur in 70-80% of all pancreatic cancers attests to their crucial role. *In vitro* studies blocking mut-RAS function in tumor cell lines with either siRNAs or by CRISPR/CAS silencing have typically resulted in widespread tumor apoptosis and non-malignant phenotypic changes. In colon cancer, detecting mutant KRAS is now an accepted and reliable biomarker for predicting the efficacy of anti-EGFR agents, again attesting to the crucial role of this oncogene in the 33% of patients in which it occurs.

The KRAS gene (Kirsten Rat Sarcoma viral oncogene homolog) encodes the KRAS protein which is a GTP-dependent kinase that controls cell division. Abnormalities in KRAS most frequently take the form of non-synonymous point mutations at codons 12, 13, and 61. KRAS is activated when it binds GTP and inactive when bound to GDP. These mutations affect the ability of KRAS to interact with the GTPase that inactivates it, resulting in constitutive activation. Because activation is a result of loss of GTPase function, it has been very difficult to develop drugs to specifically inhibit activated mutant KRAS (but not wild type KRAS), with no such agents showing clinical activity after decades of effort. Fortunately, the mutations in KRAS result in specific and consistent amino acid substitutions at codons 12, 13, and 61, with codon 12 mutations dominating. Substitutions of aspartic acid (D), valine (V), and cysteine (C) for the native glycine (G) account for the vast majority of all the abnormalities at codon 12, with codon 13 and 61 mutations comprising only a small percent of all KRAS mutations (**Table 3**). The consistency of these substitutions has led to efforts to develop T-cells that can specifically recognize the limited variety of KRAS mutations, but not wild type KRAS. Early reports of HLA-A*02:01 presented epitopes which include KRAS mutations have not been confirmed[18]. Sporadic reports of finding native T-cells recognizing other mutations presented by other HLA alleles have been reported, but they have often been relatively rare mutations or use rare HLA restrictions[19]. One recent report of TIL from a colon cancer patient showed a T-cell clonotype that recognized the G12D mutation of KRAS presented by HLA Cw0802, confirming that this mutation can be naturally immunogenic[13]. This patient had an objective regression of multiple metastases when treated with T-cells recognizing this mutant epitope. In an effort to find widely applicable reagents against mutated RAS, we searched the G12D-mutated KRAS peptide sequence from amino acids 1-23 for epitopes predicted to bind to common HLA alleles using prediction algorithms. One HLA allele predicted to bind G12D mutated epitopes in KRAS was HLA-A*11:01, so we pursued the generation of an HLA-A*11:01-restricted T-cell recognizing the G12D mutated variant of KRAS.

Table 3. *KRAS mutations in common tumors (adapted from COSMIC database).*

Tumor	Frequency of KRAS Mutations	% of All KRAS Mutations	
		G12D	G12V
Pancreatic	**70%**	**51**	**30**
Colorectal	**36%**	**34**	**24**
Lung Adeno	**20%**	**17**	**20**
Endometrial	**18%**	**36**	**24**
Ovarian (EOC)	**14%**	**41**	**37**
Prostate	**7%**	**22**	**35**

1.2.5 Generation and Testing of T-Cells and TCRs from HLA-A*11:01 Transgenic Mice that Recognize Cells Expressing G12D-Mutated KRAS[20]

Mice transgenic for the human MHC molecule HLA-A*11:01 (in chimeric form with a human constant region to allow proper engagement by the human CD8 T-cell co-receptor) were

vaccinated and boosted with KRAS G12D 7-16 peptide, VVVGADGVGK, mixed with helper peptide HBVc128-140 in incomplete Freund's adjuvant. Lymphocytes from immunized mice were stimulated *in vitro* with the same peptide and IL-2. TCR chains were cloned from cultures reactive with an HLA-A*11:01+, G12D KRAS+ target cell using 5' RACE. Two productive alpha-beta chain pairs (TRAV4-4*01/BV12-2*01 and TRAV12N-3*01/TRBV4*01) showed recognition of the mutated KRAS peptide pulsed onto HLA-A11+ cells and HLA-A11+ cells transduced with genes encoding the G12D variant of mutated KRAS. These receptors were cloned into the bicistronic pMSGV1 plasmid, as previously described[2]. Replication-deficient retroviral supernatants containing these alpha-beta TCRs were used to transduce donor PBL (**Figure 2**) and those cells were tested against a panel of target cells expressing or not expressing HLA-A*11:01 and G12D mut-KRAS. The TRAV4-4*01/BV12-2*01 receptor showed superior tumor recognition and was highly specific for recognition of HLA-A*11:01+ target cells pulsed with the G12D KRAS peptide epitope or transduced with genes incorporating G12D mutant KRAS with no recognition of wild type KRAS (**Figure 3**). When pancreatic cancer cell lines were tested (transduced with HLA-A*11:01 if not naturally expressing this allele), all were recognized to some degree, proportional to the level of expression of mut-KRAS (**Figure 3**).

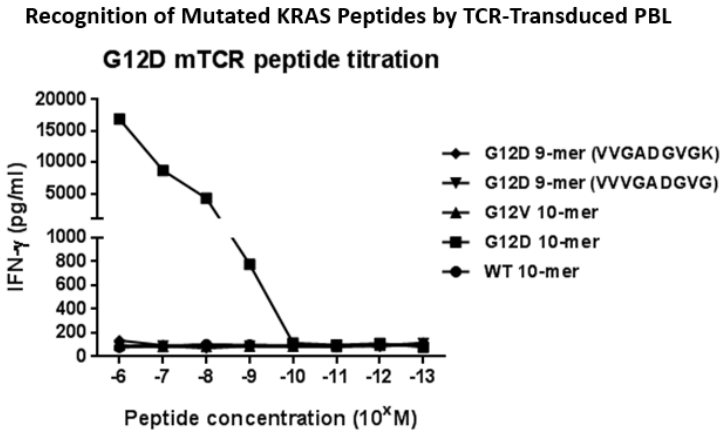


Figure 2. *Donor PBL transduced with the anti-G12D KRAS TCR were tested for recognition of 9-mer or 10-mer KRAS peptides as indicated, titered onto COS-A11. After overnight co-culture, release of IFN-gamma was measured as an indication of immune recognition. The recognized minimal determinant appears to be the aa7-16 G12D 10-mer with no recognition of the homologous G12V or wild type epitopes.*

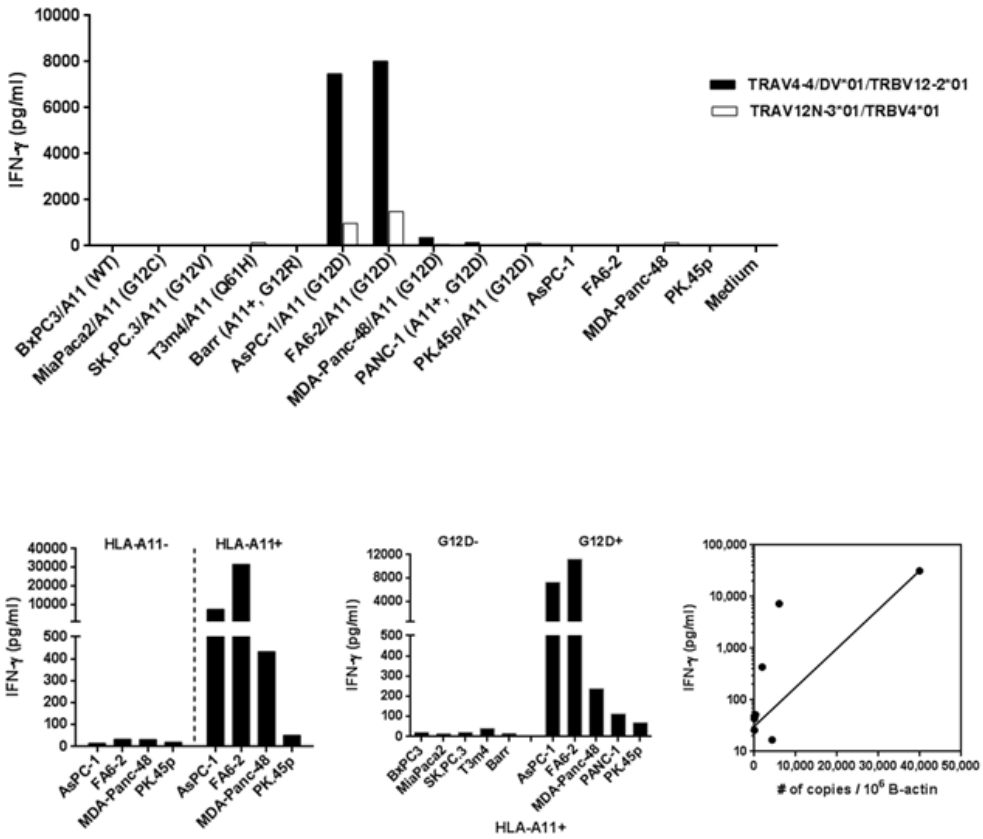


Figure 3. (Upper) *Donor PBL transduced with either of the mTCRs were tested for recognition of COS-A11 transduced with various minigenes encoding KRAS variants, as well as various tumor lines harboring a variety of KRAS variants and transduced or not transduced to express HLA-A*11:01. After overnight co-culture, release of IFN-gamma was measured as an indication of immune recognition. The TRAV4-4/TRBV12-2 receptor showed higher reactivity without recognition of HLA-A*11:01 negative target cells or targets lacking the G12D mutation.* ***(Lower)*** *Pancreatic cancer lines are grouped according to HLA-A11 expression and mutation status and tested against PBL transduced with the TRAV4-4/TRBV12-2 receptor. Again INF-γ release is measured. The right panel shows each tested HLA-A11+ line according to INF released and copies of mutated G12D as determined by mutation specific KRAS RT-PCR.*

To try to predict cross-reactivity with other human proteins, synthetic peptides substituting alanine (or glycine at position 5 where there is a native alanine) for each residue in the G12D KRAS determinant were made to determine which residues were and were not critical for TCR recognition. Amino acids 4, 5, 6, 8, 9, and 10 could not be replaced without a major reduction in recognition (data not shown). If these are fixed and any other amino acids are allowed at all the remaining positions, a BLAST search of the human proteome reveals only one protein with homology that conforms to these constraints. This was CHADL (small leucine-rich protein chondroadherin-like). The homologous CHADL peptide was made and it did not stimulate PBL transduced with the anti-G12D RAS TCR (data not shown). In addition, several normal tissues lines were procured and transduced to express HLA-A11. Human astrocytes, cardiomyocytes, and renal epithelial cells survived transduction and expressed significant levels of HLA-A11 by FACS (**Figure 4**). None of these stimulated the release of significant amounts of IFN-gamma on co-culture with PBL transduced with the anti-G12D KRAS TCR. As already shown, this TCR also shows no reactivity with wild type KRAS nor other mutated variants of KRAS, by either

peptide pulsing, mut-KRAS minigene transfection or testing against panels of HLA-A11 tumor lines.

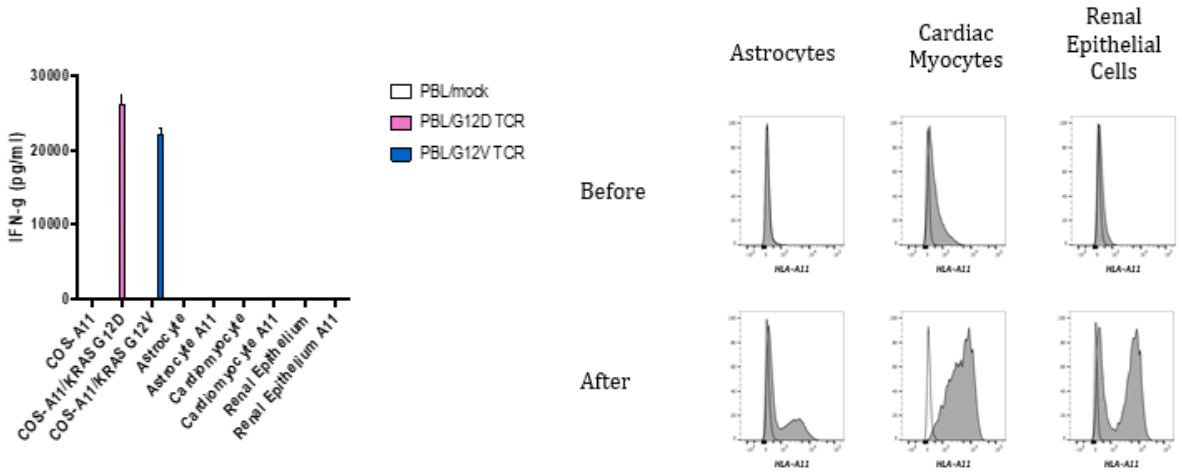


Figure 4. *Normal tissue lines were purchased and transduced with a retrovirus encoding HLA-A11. Viable astrocytes, cardiomyocytes, and renal epithelial cells were obtained that expressed HLA-A11. PBL transduced with the anti-G12D KRAS TCR or a control anti-G12V KRAS TCR were co-cultured with these normal tissue lines or COS-A11 expressing or not expressing G12D KRAS. The three normal tissue lines expressed HLA-A11 after transduction but did not release INF-γ, while the positive control COS line stimulated over 20,000 pg of INF/24 hours. Both TCRs were specific for their nominal targets.*

A master cell bank expressing the vector for this TCR was created using the PG13 packaging line, and a high producing clone was selected to make a clinical grade retroviral supernatant for clinical use. High levels of TCR transduction were achieved with this retroviral supernatant (>50% transduction), and TCR-transduced PBL from multiple donors showed specific recognition of multiple target cells expressing HLA-A*11:01 and the G12D mutated KRAS epitope but not wild type KRAS or other mutations. There was also complete specificity for HLA-A and G12D mutated tumors when a panel of tumors was interrogated (**Figure 3**).

The frequency of the HLA-A*11:01 gene in various U.S. populations is as follows (number of patients with the allele is approximately two times the frequency minus the frequency of homozygosity at the A locus):

Gene Frequencies of HLA-A Alleles in U.S. Populations[21]					
HLA-A	**African Americans (n = 252)**	**Caucasians (n = 265)**	**Hispanics (n = 234)**	**North American Natives (n = 187)**	**Asians (n = 358)**
11:01	0.0238	0.0698	0.0577	0.0267	0.2312

Although HLA-A*11:01 is present in only 13% of the population, there are over 100,000 patients in the U.S. who will die of KRAS-mutated malignancies, so we expect over 10,000 patients per year to have HLA-A*11:01 and one of the two KRAS mutations (G12V and G12D) for which we have identified TCRs. Therefore, we expect to be able to screen and enroll at least 10-15 patients per year. A sister protocol with a parallel TCR against G12V is currently enrolling patients, and accrual is likely to improve when both

protocols are open, as the primary eligibility test for RAS mutated gastrointestinal cancers will then be only HLA-A11 testing, which is simple and available.

The eligibility, design, treatment, and evaluation of patients on this protocol will be similar to what has been used in studies that have enrolled over 400 patients with a variety of T-cells engineered with a retrovirally-introduced antigen receptor. Many safety concerns regarding the infusion of large numbers of retrovirally-modified tumor reactive T-cells have been addressed in our previous clinical studies. The non-myeloablative chemotherapy and the administration of high-dose aldesleukin have expected toxicities, which are discussed in Section **8.4**. The immunosuppressive chemotherapy used in this protocol has been administered to over 800 patients and patients have not required stem cell support to reconstitute their hematopoietic systems. In other protocols, we have administered up to $1.5x10^{11}$ TIL with widely heterogeneous reactivity, including CD4, CD8, and NK cells, without difficulty. As discussed above, the expansion of tumor reactive cells is a desirable outcome following the infusion of antigen reactive T-cells. We do not believe the transfer of these gene-modified cells poses a significant risk for malignant transformation in this patient population. While the risk of insertional mutagenesis is a known possibility using retroviral vectors, this has only been observed in the setting of infants treated for XSCID using retroviral vector-mediated gene transfer into CD34+ bone marrow cells. In the case of retroviral vector-mediated gene transfer into mature T-cells, there has been no evidence of long-term toxicities associated with these procedures since the first NCI-sponsored gene transfer study in 1989. Although continued follow-up of all gene therapy patients will be required, data suggest that the introduction of retroviral vectors transduced into mature T-cells is a safe procedure. While we believe the risk of insertional mutagenesis is extremely low, the proposed protocol follows all current FDA guidelines regarding testing and follow-up of patients receiving gene-transduced cells. Murine TCRs have been used in six previous TCR gene therapy trials in the NCI-SB (04-C-0241, 07-C-0003, 07-C-0174, 09-C-0047, 11-C-0062, 13-C-0214). The introduction of murine or murinized TCRs and the possibility of immune responses against murine antigens has been studied in two previous NCI-SB clinical trials in which cancer patients were treated with mTCRs specific for the antigens p53 and gp100; these studies found that 23% of patients treated with the mTCRs developed antibodies directed towards the murine variable regions and not to the constant region common to all mTCRs[22]. These antibodies were not detected until 3-4 months after cell transfer and the production of these antibodies was not associated with specific toxicity, altered levels of transduced cell persistence, or fewer responses to therapy.

1.2.6 Safety Considerations

T-cells engineered with retroviruses encoding antigen-reactive receptors have been given to over 500 patients in the NCI-SB and hundreds of additional patients worldwide. There are several types of risks encountered. The most frequent source of mortality and morbidity in our experience has been from the preparative regimen of cyclophosphamide and fludarabine. Sepsis in the presence of neutropenia accounts for a mortality of approximately one percent[23]. Bleeding associated with thrombocytopenia is very rare, even when patients have had small brain metastases. Other very rare toxicities of this chemotherapy can include neurotoxicity from fludarabine or cardiac injury from cyclophosphamide. Another source of toxicity has been cytokine release syndrome (CRS) with fever, vascular leak, hypotension, and potential multiorgan failure. This can occur with any immunotherapy and to varying degrees; it was uniformly encountered when patients were treated with high-dose IL-2. The NCI-SB developed

the algorithms used to deal with IL-2 induced CRS and reported on the treatment of over 1200 patients[24]. In our last 809 patients treated with high-dose IL-2, there were no treatment-related deaths. Nevertheless, CRS can require intensive supportive care, including ventilator, renal replacement, and vasopressor support, but absent mortality, it is typically totally reversible.

The remaining major toxicities of T-cell therapy are autoimmune. These can result from either "on-target, off-tumor" or "off-target" reactivity of the T-cells. Whenever normal self-antigens are targeted, the possibility of injury to a normal tissue expressing even low amounts of the antigen is present. Such events have been well described for T-cells directed at melanocytic proteins, CEA, or B-cell antigens[4, 5, 7]. In these protocols, vitiligo with uveitis, colitis, and B-cell aplasia were seen, respectively. In some cases, this was acceptable (e.g., anti-CD19 CARs), but in others it was not. A major advantage of targeting mutated RAS with a receptor that shows no recognition of wild type RAS is that such "on-target, off-tumor" toxicities should be avoided. Off-target toxicity results from the unanticipated recognition of a structurally similar epitope from another protein by the TCR being studied. Rare cases of fatal toxicity as a consequence of off-target recognition have been described. An HLA-A2 restricted TCR recognizing an epitope in MAGE-A3 cross-recognized the highly homologous MAGE-A12 protein, expressed in areas of the brain, leading to irreversible coma[25]. A highly modified TCR targeting an HLA-A1 presented epitope of MAGE-A3 was shown to have acquired reactivity against the cardiac protein titin, leading to fatal cardiac toxicity[10]. Although methods have been described to screen for such off-target recognition[10] and extensive modifications of native TCRs are being avoided, there are no tests that can detect all possible cross-reactivities of a TCR. In the NCI-SB's experience with over 500 patients in adoptive therapy protocols with gene-engineered T-cells testing over a dozen different receptors, the overall mortality from all causes has been between 1-2%.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Measurable (per RECIST v1.1 criteria), metastatic, or unresectable malignancy expressing G12D mutated KRAS as assessed by one of the following methods: RT-PCR on tumor tissue, tumor DNA sequencing, or any other CLIA-certified laboratory test on resected tissue. Patients shown to have tumors expressing G12D mutated NRAS and HRAS will also be eligible as these oncogenes share complete amino acid homology with G12D mutated KRAS for their first 80 N-terminal amino acids, completely encompassing the target epitope.

2.1.1.2 Patients must be HLA-A*11:01 positive as confirmed by the NIH Department of Transfusion Medicine.

2.1.1.3 Confirmation of the diagnosis of cancer by the NCI Laboratory of Pathology.

2.1.1.4 Patients must have:

2.1.1.4.1 previously received standard systemic therapy for their advanced cancer and have been either non-responders or have recurred, specifically:

- Patients with metastatic colorectal cancer must have had at least two systemic chemotherapy regimens that include 5FU, leucovorin, bevacizumab,

oxaliplatin, and irinotecan (or similar agents), or have contraindications to receiving those medications.

- Patients with pancreatic cancer must have received gemcitabine, 5FU, and oxaliplatin (or similar agents), or have contraindications to receiving those medications.
- Patients with non-small cell lung cancer (NSCLC) must have had appropriate targeted therapy as indicated by abnormalities in ALK, EGFR, or expression of PDL-1. Other patients must have had platinum-based chemotherapy.
- Patients with ovarian cancer or prostate cancer must have had approved first-line chemotherapy.

OR

2.1.1.4.2 declined standard treatment.

2.1.1.5 Patients with 3 or fewer brain metastases that are < 1 cm in diameter and asymptomatic are eligible. Lesions that have been treated with stereotactic radiosurgery must be clinically stable for one month after treatment for the patient to be eligible. Patients with surgically resected brain metastases are eligible.

2.1.1.6 Age ≥ 18 years and ≤ 72 years.

2.1.1.7 Clinical performance status of ECOG 0 or 1 (see **Appendix A**).

2.1.1.8 Patients must be willing to practice birth control from the time of enrollment on this study and for 12 months after the last dose of combined chemotherapy for women and for four months after treatment for men.

2.1.1.9 Women of child-bearing potential must be willing to undergo a pregnancy test prior to the start of treatment because of the potentially dangerous effects of the treatment on the fetus.

NOTE: Certain malignancies may secrete hormones that produce false positive pregnancy tests. Serial blood testing (e.g. HCG measurements) and/ or ultrasound may be performed for clarification.

2.1.1.10 Serology

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

2.1.1.11 Hematology

- ANC > 1000/mm^3 without the support of filgrastim
- WBC ≥ 2500/mm^3

- Platelet count ≥ 80,000/mm^3
- Hemoglobin > 8.0 g/dL. Subjects may be transfused to reach this cut-off.

2.1.1.12 Chemistry

- Serum ALT/AST ≤ 5.0 x ULN
- Serum creatinine ≤ 1.6 mg/dL
- Total bilirubin ≤ 2.0 mg/dL, except in patients with Gilbert's Syndrome, who must have a total bilirubin < 3.0 mg/dL.

2.1.1.13 Patients must have completed any prior systemic therapy at the time of enrollment.

Note: Patients may have undergone minor surgical procedures or limited field radiotherapy within the four weeks prior to enrollment, as long as related major organ toxicities have recovered to ≤ grade 1.

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

2.1.1.15 Willing to sign a durable power of attorney.

2.1.1.16 Subjects must be co-enrolled on the protocol 03C0277.

2.1.2 Exclusion Criteria

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

2.1.2.2 Concurrent systemic steroid therapy.

2.1.2.3 Active systemic infections requiring anti-infective treatment, coagulation disorders, or any other active or uncompensated major medical illnesses.

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. Patients who have decreased immune-

competence may be less responsive to the experimental treatment and more susceptible to its toxicities.)

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

2.1.2.7 History of coronary revascularization or ischemic symptoms.

2.1.2.8 For select patients with a clinical history prompting cardiac evaluation: last known LVEF ≤ 45%.

2.1.2.9 For select patients with a clinical history prompting pulmonary evaluation: known FEV1 ≤ 50%.

2.1.2.10 Patients who are receiving any other investigational agents.

2.1.3 Recruitment Strategies

This study will be posted on clinicaltrials.gov, NIH websites, and on NIH social media forums.

2.2 SCREENING EVALUATION

Note: Testing for screening evaluation is conducted under the NCI-SB screening protocol, 99C0128 (Evaluation for NCI Surgery Branch Clinical Research Protocols). Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility.

2.2.1 At Any Time Prior to Enrollment

- HIV antibody titer, HBsAg determination, and anti-HCV (Note: All patients will have values from the time of enrollment on the required companion protocol 03C0277. Repeat testing may be performed prior to treatment.)
- Anti-CMV antibody titer, HSV and VZV serology, and EBV panel (Note: Patients who are known to be positive do not need to be retested.)
- Confirmation of HLA-A*11:01 positivity by the NIH Department of Transfusion Medicine.
- Confirmation of KRAS, NRAS, or HRAS G12D by a CLIA-certified laboratory.
- Confirmation of the diagnosis of cancer by the NCI Laboratory of Pathology. (Note: Testing is permitted to be conducted at any time prior to enrollment.)
- For select patients: Pulmonary function testing for patients with a prolonged history of cigarette smoking (≥ 20 pack-year smoking history, with cessation within the past two years), symptoms of respiratory dysfunction, or other clinical indications which may include thoracic surgeries.
- For select patients: Cardiac evaluation commensurate to patients' history and clinical presentation (e.g., stress thallium, echocardiogram, MUGA) for patients who are ≥ 65 years of age, 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. Patients with a LVEF ≤ 45% will not be

eligible. Patients < 65 years of age with cardiac risk factors (e.g., diabetes, hypertension, obesity) may undergo cardiac evaluation as noted above.

2.2.2 Within 6 Weeks Prior to Enrollment

- History and physical examination, including weight and vital signs, noting organ system involvement and any allergies/sensitivities to antibiotics. (Note: History may be documented via telehealth used in compliance with local policy and physical exam may incorporate medical documentation from non-NIH physicians/physician extenders. Will be repeated at baseline prior to treatment.)
- Imaging evaluation to determine baseline disease status. This may include CT, MRI, PET, and/or photography.

2.2.3 Within 14 Days Prior to Enrollment

- Screening blood tests:
  - CBC w/differential
  - Chemistries: Creatinine, ALT/GPT, AST/GOT, Total bilirubin
- Urinalysis, with culture if indicated
- ECOG performance status of 0 or 1 (see **Appendix A**).

2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE

2.3.1 Prior to Registration for this Protocol

Patients will sign the consent for and enroll on protocol 03C0277 prior to leukapheresis for generation of the cell product. Patients will sign the consent document for this protocol prior to the start of cell product manufacturing.

2.3.2 Participant Registration and Status Update Procedures

Registration and status updates (e.g., when a patient is taken off protocol therapy and when a patient 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.

2.3.3 Treatment Assignment Procedures

2.3.3.1 Cohorts

Number	Name	Description
1	Phase I KRAS G12D	First 12-36 patients enrolled with measurable, metastatic, or unresectable malignancy expressing G12D mutated KRAS
2a	Phase II Pancreatic Cancer	Patients with a diagnosis of pancreatic cancer
2b	Phase II Non-Pancreatic Cancer	Patients with a diagnosis other than pancreatic cancer

2.3.3.2 Arms

Number	Name	Description
1	Phase I	Non-myeloablative, lymphodepleting preparative regimen of cyclophosphamide and fludarabine + escalating doses of anti-KRAS G12D mTCR PBL + high-dose aldesleukin
2	Phase II	Non-myeloablative, lymphodepleting preparative regimen of cyclophosphamide and fludarabine + MTD of anti-KRAS G12D mTCR PBL + high-dose aldesleukin

2.3.3.3 Randomization and Arm Assignment

This is a non-randomized study. All patients will be directly assigned based on cohort as follows:

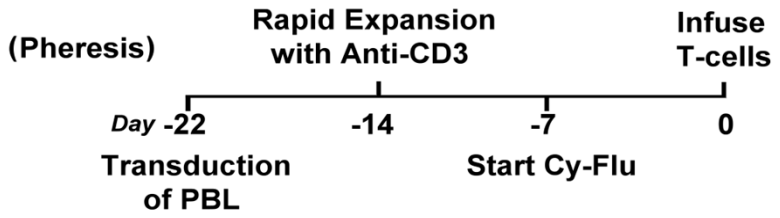
- Patients in Cohort 1 will be directly assigned to Arm 1.
- Patients in Cohort 2a will be directly assigned to Arm 2.
- Patients in Cohort 2b will be directly assigned to Arm 2.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

3.1.1 Study Treatment Timeline

The timeline for apheresis, cell production, and treatment is as follows:



3.1.2 Pre-Treatment Phase: Manufacturing

PBMC will be obtained by leukapheresis (approximately 0.2-1x10^{10} cells). Whole PBMC will be cultured in the presence of anti-CD3 (OKT3) and aldesleukin to stimulate T-cell growth. Transduction is initiated by exposure of approximately 1x10^{7} to 5x10^{8} cells to supernatant containing the anti-KRAS G12D 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 murine TCR protein and anti-tumor reactivity will be tested by cytokine release as measured on peptide pulsed COS A11 cells. Successful TCR gene transfer for each transduced PBL population will be defined as > 10% TCR-positive cells and for biological activity, gamma-interferon secretion ≥ 200 pg/mL, and ≥ 2 times background level.

3.1.3 Treatment Phase

Transduced PBL must be projected to meet the release criteria as specified in the Certificate of Analysis found in BB-IND 18470.

Patients will receive the standard NCI-SB non-myeloablative, lymphodepleting preparative regimen consisting of cyclophosphamide and fludarabine, and will then receive high-dose

intravenous aldesleukin to tolerance after cells are infused. All patients will receive one course of treatment. The start date of the course will be the start date of the chemotherapy; the end date will be the day of the first post-treatment evaluation.

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 end two weeks after cell infusion. A DLT is defined as follows: All grade 3 and greater toxicities related to the cell infusion, **with the exception of**:

- Myelosuppression, defined as lymphopenia, neutropenia, decreased hemoglobin, and thrombocytopenia, due to the non-myeloablative, lymphodepleting preparative regimen.
- Expected chemotherapy toxicities as defined in Section **14**.
- Aldesleukin expected toxicities as defined in **Appendix B** and **Appendix C**.
- Immediate hypersensitivity reactions (excluding symptomatic bronchospasm and grade 4 hypotension) occurring within two hours of cell infusion (related to cell infusion) that are reversible to a grade 2 or less within 24 hours of cell administration with standard therapy.
- Grade 3 fever
- Grade 3 metabolic laboratory abnormalities without significant clinical sequela that resolve to grade 2 within 7 days.
- Grade 3 autoimmune toxicity that resolves to grade 2 or less within 10 days, unless immunosuppression is required.
- Events that are clearly related to the patient's disease.

3.1.5 Phase I – Dose Escalation

Initially, patients will be entered in sequential dose levels and receive escalating doses of cells beginning at $1x10^8$ and increasing approximately three-fold per dose level. The protocol will initially enroll one patient in each dose level in the first three dose levels and then three per dose level starting with $3x10^9$ cells, unless a patient experiences a DLT. The maximum total number of anti-KRAS G12D mTCR engineered PBL cells infused at each dose level (± 25%) will be:

Dose Level	**Cell Dose***
Level 1 (Completed)	$1x10^8$ cells
Level 2 (Completed)	$3x10^8$ cells
Level 3 (Completed)	$1x10^9$ cells
Level 4	$3x10^9$ cells
Level 5	$3x10^{10}$ cells
Level 6	Up to $1.5x10^{11}$ cells

[*total number of TCR-transduced cells]

As of Amendment D, the study will have treated a total of four patients with cell doses up to $3x10^9$. The first patient treated at Dose Level 4 experienced grade 3 diarrhea with subsequent hospitalization, possibly related to the cell infusion, which is a DLT per protocol. No other patients have experienced a DLT and only expected adverse events related to the experimental treatment have been seen. The greatest risk seen with the NCI-SB T-cell protocols has been the preparative regimen treated patients receive (regardless of cell dose), which causes a 7-10 day period of total neutropenia and thrombocytopenia. This has caused the majority of morbidity and mortality across the NCI-SB adoptive T-cell protocols. Experience with solid tumors such as melanoma and synovial sarcoma indicates 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 patients may be exposed to the risks of the preparative regimen before achieving optimal cell numbers. To avoid this, an escalation of 5-10 fold per dose level starting at $3x10^9$ cells will be used up to a maximum cell dose of $1.5x10^{11}$, with at least three patients treated per dose level. The parallel NCI-SB protocol targeting the G12V mutation in KRAS with TCR-engineered PBL has been approved with 10x cell dose escalations per dose level up to the same maximum cell dose.

In each dose level, if a patient experiences a DLT, a total of six patients will be treated at that dose to confirm that no greater than 1 of 6 patients have a DLT prior to proceeding to the next higher dose level. If a level with two or more DLTs in six patients has been identified, patients will be accrued at the next-lowest dose, for a total of six patients.

The MTD is the highest dose at which ≤ 1 of 6 patients experienced a DLT or the highest dose level studied if DLTs are not observed at any of the dose levels. If a patient did not experience a DLT and did not finish treatment, he or she will not be evaluable for toxicity and will be replaced in the dose level.

Patients for whom the designated number of cells cannot be generated will be treated at the appropriate dose level for the number of cells generated. Toxicities seen in these patients will not be used in determining the MTD.

3.1.6 Safety Assessment

A two-week safety assessment period following cell administration will be conducted between each dose level and before enrollment into the Phase II portion of the protocol.

3.1.7 Phase II

Similar to the Phase I portion, prior to receiving the engineered PBL cells, patients in the Phase II portion will receive a non-myeloablative, lymphodepleting preparative regimen consisting of cyclophosphamide and fludarabine followed in two to four days by intravenous infusion of anti-KRAS G12D mTCR PBL.

The Phase II portion of the protocol will consist of two cohorts as defined in Section **2.3.3.1**: Cohort 2a, patients with RAS G12D pancreatic cancer, and Cohort 2b, patients with RAS G12D non-pancreatic cancer.

The Phase II portion of the protocol will proceed using up to the MTD as determined in the Phase I portion of the study.

The Phase II portion of the study will utilize a Simon minimax design where initially 12 evaluable patients will be enrolled in each cohort. For each cohort, if 0 of the first 12 evaluable

patients experiences a clinical response, then no further patients will be enrolled; if 1 or more of the first 12 evaluable patients enrolled have a clinical response, then accrual will continue until a total of 21 evaluable patients have been enrolled in that cohort.

Note: As stated in Section **10.2**, up to 6 patients enrolled at the MTD will count towards the accrual in the Phase II portion of the trial if they are evaluable for response and if they would be fully eligible for enrollment in the Phase II portion of the trial.

3.2 PROTOCOL STOPPING RULES

New subject enrollment to the protocol will be temporarily halted if any of the following conditions are met, and consideration will be given to the need for protocol revisions regarding safety, if applicable. Consultation with the FDA may be arranged by the Sponsor before or after the protocol amendment is submitted to the NIH Intramural IRB, as needed.

- During the Phase I portion of the study – If two DLTs, as described in Section **3.1.4**, occur in Dose Level 1.
- During the Phase I portion of the study – If two or more patients develop a grade 3 or greater toxicity related to the cell product, with the exception of:
  - Grade 3 metabolic laboratory abnormalities without significant clinical sequela that resolve to grade 2 or less within 7 days.
  - Grade 3 fever
- If 2 of the first 6 patients (OR 3 of the first 9 patients, OR 4 of the first 12 patients) develop:
  - Grade 3 autoimmune toxicity that cannot be resolved to grade 2 or less within 10 days.
  - Any grade 4 or greater autoimmune toxicity.
- During the Phase II portion of the study – Once five or more patients have been enrolled, if 20% or more patients cumulatively enrolled develop a DLT, as described in Section **3.1.4**.
- During the Phase I and II portions of the study – Any death that is at least possibly attributed to the investigational agent and which occurs within 30 days of receiving the investigational agent.

3.3 DRUG ADMINISTRATION

3.3.1 Preparative Regimen with Cyclophosphamide and Fludarabine

Treatment will be according to the schedule described below and in Section **3.3.4**. Chemotherapy infusions may be slowed or delayed as medically indicated. In Dose Level 6, unplanned dose reductions are allowed due to clinical indications and will be discussed with the PI and documented in CRIS before implementation. Administration of diuretics, electrolyte replacement, and hydration and monitoring of electrolytes should all be performed as clinically indicated.

Days -7 and -6

Approximately 6 Hours Prior to Cyclophosphamide

Hydrate: Begin hydration with 0.9% Sodium Chloride Injection containing 20 mEq/L of potassium chloride at 1.5-2.0 mL/kg/hour (starting approximately 6 hours pre-cyclophosphamide and continuing until 24 hours after last cyclophosphamide infusion). Rate and composition of fluid may be altered based on clinical indications. The hydration rate will be capped at 250 mL/hour. At any time during the preparative regimen, if urine output is < 1.0 mL/kg/hour or if body weight > 2 kg over pre-cyclophosphamide value, furosemide 10-20 mg IV may be administered.

Approximately 1 Hour Prior to Cyclophosphamide

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

Cyclophosphamide 60 mg/kg/day x 2 days IV in 250 mL D5W infused simultaneously with mesna 15 mg/kg/day over one hour x 2 days. If the patient is obese (BMI > 35), drug dosage will be calculated using practical weight as described in **Appendix D**.

For Dose Level 6, a decreased dose of cyclophosphamide at 30 mg/kg/day (x 2 days) will be considered for patients who have a history of prolonged hematologic recovery from prior chemotherapy treatments. A reduction in cyclophosphamide will be accompanied by a reduction in concomitant mesna to 7.5 mg/kg/day over one hour x 2 days.

Immediately Following the End of Cyclophosphamide

Begin mesna infusion at 3 mg/kg/hour intravenously diluted in a suitable diluent (see Section **14.2.1**) over 23 hours after each cyclophosphamide dose. If decreased dose cyclophosphamide of 30 mg/kg/day is used, the corresponding mesna dose should be reduced to 1.5 mg/kg/hour. If the patient is obese (BMI > 35), drug dosage will be calculated using practical weight as described in **Appendix D**.

Days -7 to -3

Fludarabine 25 mg/m^2/day IVPB daily over 30 minutes for 5 days. If the patient is obese (BMI > 35), drug dosage will be calculated using practical weight as described in **Appendix D**. Fludarabine will be started approximately 1-2 hours after the cyclophosphamide and mesna on Days -7 and -6.

3.3.2 Cell Infusion

Day 0 (2-4 Days After the Last Dose of Fludarabine)

The patient's PBMC transduced cells will be delivered to the Patient Care Unit by a staff member from the NCI-SB. Prior to infusion, the cell product identity label is double-checked by two authorized staff members (MD or RN), and an identification of the product and documentation of administration are entered in the patient's chart. The cells will be infused intravenously on the Patient Care Unit over 20-30 minutes or as clinically determined by an investigator for patient safety via non-filtered tubing, gently agitating the bag during infusion to prevent cell clumping.

3.3.3 Aldesleukin Administration

Days 0-3 (Day 0 = Day of Cell Infusion)

- Beginning on Day 1, filgrastim will be administered subcutaneously at a dose of 300 mcg/day). Dose and route of administration may be altered as clinically indicated. Filgrastim administration will continue daily until ANC > 1000/mm^3 x 3 days or ANC > 5000/mm^3.
- Aldesleukin will be given as described below.

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 (± 5 minutes) approximately every 8 hours beginning within 24 hours of cell infusion and continuing for up to 3 days (maximum 9 doses). Doses will be preferentially administered every eight hours; however, up to 24 hours may elapse between doses depending on patient tolerance. Aldesleukin dosing will be stopped if toxicities are not sufficiently recovered with supportive measures within 24 hours of the last dose of aldesleukin. Doses will be delayed or stopped if patients reach grade 3 or 4 toxicity due to aldesleukin, except for the reversible grade 3 toxicities common to aldesleukin such as diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes as detailed in **Appendix B**. Toxicities will be managed as outlined in **Appendix C**. Dosing may be held or stopped at the discretion of the treating investigator. (**Appendix E** lists the toxicities seen in patients treated with aldesleukin at the NIH Clinical Center.)

Because confusion is a possible side effect of aldesleukin administration, a Durable Power of Attorney will be signed by the patient to identify a surrogate to make decisions about their medical care if they become incapacitated or cognitively impaired.

3.3.4 Treatment Schedule

Day	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4
Therapy												
Cyclophosphamide (60 mg/kg)	X	X										
Fludarabine (25 mg/m^2)	X	X	X	X	X							
Anti-KRAS G12D mTCR transduced PBL								X[1]				
Aldesleukin (720,000 IU/kg)								X[2]	X	X	X	
Filgrastim[3] (300 mcg/day)									X	X	X	X
TMP/SMX[4] (160 mg/800 mg (example))								X		X		X
Fluconazole[5] (400 mg PO)								X	X	X	X	X
Valacyclovir PO or Acyclovir IV[6]								X	X	X	X	X

[1]Two to four days after the last dose of fludarabine.

[2]Initiate within 24 hours after cell infusion.

[3]Continue daily until ANC > 1000/mm^3 for three consecutive days or ANC > 5000/mm^3.

[4]The TMP/SMX schedule should be adjusted to QD three times per week (Monday, Wednesday, Friday) and continue for at least six months and until CD4 > 200 x2, starting Day 0 or within one week of anticipated lymphopenia.

[5]Continue until ANC > 1000/mm^3.

[6]In patients positive for HSV or VZV, continue for at least six months and until CD4 > 200 x2.

3.4 BASELINE EVALUATIONS

Note: Refer to Section **5.4** for the research biospecimen collection schedule.

Baseline evaluations will be performed within 14 days prior to starting the preparative regimen.

- Apheresis, as indicated
- Baseline blood tests:
  - CBC w/differential
  - Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric acid, Creatine kinase, Lactate dehydrogenase, Total protein
  - PT-INR/PTT
  - TBNK
  - Thyroid Panel
  - Anti-CMV antibody titer, HSV and VZV serology, and EBV panel (if greater than three months have elapsed since last negative assessment. Note: Patients who are known to be positive do not need to be retested.)
  - HIV antibody titer, HbsAg determination, and anti-HCV (if greater than three months have elapsed since last assessment).

The amount of blood to be drawn is expected to be about 8 tablespoons. This includes testing for standard of care tests (i.e., complete blood counts) as well as blood for research.

- β-hCG pregnancy test (serum or urine) on all women of child-bearing potential
  - **NOTE**: In the rare event that the participants has a malignancies that secretes hormones that produce false positive pregnancy tests, serial blood testing (e.g. HCG measurements) and/ or ultrasound may be performed for clarification.
- Urinalysis, with culture if indicated
- Chest x-ray
- EKG
- Complete history and physical examination including weight and vital signs, ECOG, noting organ system involvement and any allergies/sensitivities to antibiotics.

3.5 ON-STUDY EVALUATIONS

3.5.1 During the Preparative Regimen (Daily)

- CBC w/differential
- Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric acid, Creatine kinase, Lactate dehydrogenase, Total protein
- PT-INR/PTT (every 3 days)
- Thyroid Panel (every 7 days)
- Urinalysis, as needed
- Weight, as indicated

3.5.2 Post-Cell Infusion

- Vital signs will be monitored before and after cell infusion then hourly (± 15 minutes) for four hours and then routinely (every 4-6 hours) unless otherwise clinically indicated.

3.5.3 During Hospitalization (Every 1-2 Days)

- Physical examination, including weight and vital signs, as clinically indicated
- Toxicity assessment, including a review of systems, as clinically indicated
- CBC w/differential
- Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric acid, Creatine kinase, Lactate dehydrogenase, Total protein
- PT-INR/PTT (every 3 days)
- Once total lymphocyte count is > 200/mm^3, TBNK for peripheral blood CD4 count will be drawn weekly (while the patient is hospitalized).
- Thyroid Panel (every 7 days)
- Urinalysis, as indicated
- Other tests will be performed as clinically indicated.

The most amount of blood to be drawn at any one time during the On-Study time period is expected to be about 6 tablespoons. This includes testing for standard of care tests (i.e., complete blood counts) as well as blood for research.

3.6 POST-TREATMENT (FOLLOW-UP) EVALUATIONS

- All patients will return to the NIH Clinical Center for their first follow-up evaluation for response 6 weeks (± 2 weeks) following the administration of the cell product.

- Patients who have received multiple transfusions during the treatment phase or have been discharged with grade 3 or greater significant adverse events should be evaluated by their local physician within two weeks of discharge and repeat labs drawn as appropriate to be faxed to the research team. Patients will receive appropriate treatment as determined by their local physician.
- Patients who are unable or unwilling to return for follow-up evaluations may be followed via phone or email contact. A request will be made to send laboratory, imaging, and physician exam reports performed by their local physician. Any outstanding toxicities will be reviewed with the patient.

3.6.1 Time-Period of Evaluations

Patients who experience stable disease, a partial response, or a complete response, or have unresolved toxicities after their first follow-up evaluation, will return to the NIH Clinical Center as noted below:

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

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

3.6.2 Scheduled Evaluations

At each scheduled evaluation for response, patients will undergo:

- Physical examination, including weight and vital signs
- Toxicity assessment, including a review of systems
- ECOG performance status
- CBC w/differential
- Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric acid, Creatine kinase, Lactate dehydrogenase, Total protein
- PT-INR/PTT
- TBNK, until CD4 > 200 x2
- Thyroid Panel, as clinically indicated

The most amount of blood to be drawn at any one time during Follow-up visit is expected to be about 3 tablespoons. This includes testing for standard of care tests (i.e., complete blood counts) as well as blood for research.

- Urinalysis, as needed

- Imaging studies as per screening assessment. If clinically indicated, other scans or x-rays may be performed, e.g., CT, MRI, and/or PET.
- A 5-liter apheresis may be performed at the first follow-up visit. If the patient is unable to undergo apheresis, approximately 96 mL of blood may be obtained. Subsequently, approximately 60 mL of blood will be obtained at follow-up visits for at least 3 months. Peripheral blood mononuclear cells (PBMC) will be cryopreserved so that immunologic testing may be performed. This will be performed on protocol 03C0277.
- Detection of RCR and persistence of TCR gene transduced cells (see Section **5.6**). This will be performed on protocol 09C0161.
- Long-term follow-up of patients receiving gene transfer: Physical examinations will be performed and documented annually for 5 years following cell infusion to evaluate long-term safety. After 5 years, health status data will be obtained from surviving patients via telephone contact. The long-term follow-up period for retroviral vectors is 15 years. This will be performed on the NCI-SB long-term follow-up protocol, 09C0161.

3.7 STUDY ASSESSMENT CALENDAR

Assessments	Screening Evaluation			Baseline - Within 14 Days Prior to Preparative Regimen	During Preparative Regimen *(Daily)*	Prior to Cell Infusion	Post-Cell Infusion	During Hospitalization *(Every 1-2 Days)*	Post-Treatment Follow-up[1]
	Any Time Prior to Enrollment	Within 6 Weeks	Within 14 Days Prior to Enrollment						
Confirmation of cancer diagnosis by NCI Lab of Pathology[2]	X								
Confirmation of HLA-A*11:01 positivity by NIH DTM[2]	X								
Confirmation of KRAS, NRAS, or HRAS G12D by CLIA-certified lab[2]	X								
Medical history[3]		X		X					
Physical exam		X		X				X[4]	X[5]
Performance status (ECOG)[6]			X[7]	X					X
Weight		X		X	X[4]			X[4]	X
Vital signs		X		X			X[7]	X[4]	X
β-HCG pregnancy test[8]				X					
Urinalysis[9]			X	X	X[4]			X[4]	X[4]
Pulmonary function test[10]	X								
Cardiac evaluation[11]	X								
EKG				X					
Toxicity assessment[12]								X[4]	X
Serology									
HIV antibody titer, HBsAg, anti-HCV	X			X					
Anti-CMV antibody titer, HSV and VZV, EBV panel[13]	X			X					
Laboratory Procedures									
CBC w/differential			X	X	X			X	X
Blood chemistries[14]			X	X	X			X	X
PT-INR/PTT				X	X[15]			X[15]	X
TBNK				X				X[16]	X[17]
Thyroid Panel				X	X[18]			X[18]	X[4]
Additional apheresis[19]				X[4]					X

Assessments	Screening Evaluation			Baseline - Within 14 Days Prior to Preparative Regimen	During Preparative Regimen *(Daily)*	Prior to Cell Infusion	Post-Cell Infusion	During Hospitalization *(Every 1-2 Days)*	Post-Treatment Follow-up[1]
	Any Time Prior to Enrollment	Within 6 Weeks	Within 14 Days Prior to Enrollment						
Persistence and RCR[20]									X
Correlatives[21]									
CPT tubes (SB-CPF)				X			X		
SST tubes (BPC)				X		X	X		
EDTA tubes (BPC)				X		X	X		
Imaging									
CT, MRI, PET, and/or photography		X[22]							X[23]
Chest x-ray				X					

[1] All patients will return to the NIH Clinical Center for their first follow-up evaluation for response 6 weeks (± 2 weeks) following the administration of the cell product. Patients who experience stable disease, a partial response, or a complete response, or have unresolved toxicities after their first follow-up evaluation, will return to the NIH Clinical Center at week 12 (± 2 weeks), every 3 months (± 1 month) x3, every 6 months (± 1 month) x 2 years, and then per PI discretion for subsequent years. Patients may be seen more frequently as clinically indicated. See Section **3.6**.
[2] Testing is permitted to be conducted at any time prior to enrollment.
[3] Note organ system involvement and any allergies/sensitivities to antibiotics. Patient history may be obtained within 6 weeks prior to enrollment.
[4] As needed or clinically indicated.
[5] Physical examinations will be performed and documented annually for 5 years following cell infusion to evaluate long-term safety. After 5 years, health status data will be obtained from surviving patients via telephone contact . The long-term follow-up period for retroviral vectors is 15 years. This will be performed on protocol 09C0161.
[6] ECOG of 0 or 1 (see **Appendix A**).
[7] Vital signs will be monitored before and after cell infusion then (± 15 minutes) for four hours and then routinely (every 4-6 hours) unless otherwise clinically indicated.
[8] Serum or urine; on all women of child-bearing potential. In the rare event that the participants has a malignancies that secretes hormones that produce false positive pregnancy tests, serial blood testing (e.g. HCG measurements) and/ or ultrasound may be performed for clarification.
[9] With culture if indicated.
[10] For patients with a prolonged history of cigarette smoking (≥ 20 pack-year smoking history, with cessation within the past two years), symptoms of respiratory dysfunction, or other clinical indications which may include thoracic surgeries.
[11] Commensurate to patients' history and clinical presentation (e.g., stress thallium, echocardiogram, MUGA) for patients who are ≥ 65 years of age, 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. Patients with a LVEF ≤ 45% will not be eligible. Patients < 65 years of age with cardiac risk factors (e.g., diabetes, hypertension, obesity) may undergo cardiac evaluation as noted above.
[12] Including a review of systems.
[13] Patients who are known to be positive do not need to be retested. May be performed within 3 months prior to starting the preparative regimen.
[14] **Screening:** Creatinine, ALT/GPT, AST/GOT, Total bilirubin. **All other times:** Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric acid, Creatine kinase, Lactate dehydrogenase, Total protein.
[15] Every 3 days.
[16] Once total lymphocyte count is > 200/mm^3, TBNK for peripheral blood CD4 count will be drawn weekly (while the patient is hospitalized).
[17] Until CD4 > 200 x2.
[18] Every 7 days.
[19] Apheresis may be performed prior to and 6 weeks (± 2 weeks) following the administration of the cell product. If the patient 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 3 months. PBMC will be cryopreserved so that immunologic testing may be performed. This will be performed on protocol 03C0277.
[20] Detection of RCR and persistence of TCR gene transduced cells (see Section **5.6**). This will be performed on protocol 09C0161.
[21] Research samples, as described in Section **5.4**.
[22] Imaging evaluation to determine baseline disease status.
[23] Imaging studies as per screening assessment. If clinically indicated, other scans or x-rays may be performed.

3.8 COST AND COMPENSATION

3.8.1 Costs

NIH does not bill health insurance companies or patients for any research or related clinical care that patients receive at the NIH Clinical Center. If some tests and procedures are performed outside the NIH Clinical Center, patients may have to pay for these costs if they are not covered by their insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

3.8.2 Compensation

Patients will not be compensated on 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 patient/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 an evaluation safety visit approximately 6 weeks (± 2 weeks) following administration of the cell product (at the first follow-up evaluation).

3.9.1 Criteria for Removal from Protocol Therapy

Patients will be taken off treatment for the following:

- Completion of first follow-up evaluation
- Progression of disease
- Patient requests to be withdrawn from protocol therapy
- Investigator discretion
- Positive pregnancy test

3.9.2 Off-Study Criteria

Patients will be taken off-study for the following:

- Completion of study follow-up period
- Progression of disease
- Patient begins a new therapy for their cancer
- Patient requests to be withdrawn from study
- Significant noncompliance
- Investigator discretion
- Death

All patients will be co-enrolled on protocol 09C0161 for long-term follow-up; lost to follow-up criteria listed in that protocol will be observed.

Once a subject is taken off study, no further data can be collected on this treatment protocol.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 INFECTION PROPHYLAXIS

Note: Other anti-infective agents may be substituted at the discretion of the treating investigator. Patients are allowed to miss 25% of their required doses of prophylactic drugs.

4.1.1 Pneumocystis Jirovecii Pneumonia

All patients will receive the fixed combination of trimethoprim and sulfamethoxazole (TMP/SMX) as double strength (DS) tab (DS tabs = TMP 160 mg/tab and SMX 800 mg/tab) PO daily three times a week on non-consecutive days, beginning Day 0 or within one week of anticipated lymphopenia.

Dapsone (in G6PD sufficient patient), atovaquone, or pentamidine may be substituted for TMP/SMX-DS in patients with sulfa allergies.

4.1.2 Herpes Simplex or Varicella Zoster Virus Prophylaxis

Patients with positive HSV or VZV serology will be given valacyclovir orally at a dose of 500 mg daily starting on the day of cell infusion, or acyclovir, 250 mg/m^2 IV every 12 hours if the patient is not able to take medication by mouth. Reversible renal insufficiency has been reported with IV but not oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs have been reported with higher doses of acyclovir. Should this occur, a dosage adjustment will be made or the drug will be discontinued. Acyclovir will not be used concomitantly with other nucleoside analogs which interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling.

Prophylaxis for pneumocystis, HSV, and VZV will continue for at least six months and until the CD4 count is > 200 for two consecutive measures.

4.1.3 Fungal Prophylaxis (Fluconazole)

Patients will start fluconazole 400 mg PO starting on the day of cell infusion and continue until ANC > 1000/mm^3. The drug may be given IV at a dose of 400 mg in 0.9% sodium chloride USP daily in patients unable to take it orally.

4.1.4 Empiric Antibiotics

Patients will start on broad-spectrum antibiotics in accordance with current institutional guidelines for fever of 38.3°C once or two temperatures ≥ 38.0°C at least one hour apart, AND an ANC < 500/mm^3. Infectious disease consultation will be obtained for all patients with unexplained fever or any infectious complications.

4.2 BLOOD PRODUCT SUPPORT

Using daily CBCs as a guide, the patient will receive platelets and packed red blood cells (PRBCs) as needed. As a general guideline, patients may be transfused for:

- Hemoglobin < 8 gm/dL

- Platelets < 10,000/mm^3

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

4.3 Other Concomitant Medications to Control Side Effects

Concomitant medications to control side effects of therapy may be given. Meperidine (25-50 mg) will be given intravenously if severe chilling develops. Other supportive therapy will be given as required and may include acetaminophen (650 mg every 4 hours), indomethacin (50-75 mg every 8 hours), and famotidine (20 mg every 12 hours) or other H2 receptor blocker of equivalent efficacy may be administered. If patients require steroid therapy, they will be taken off treatment. Patients who require transfusions will receive irradiated blood products. Ondansetron (0.15 mg/kg/dose IV every 8 hours) will be administered for nausea and vomiting. Dosing may be adjusted to administration as needed, and other antiemetics may be substituted as clinically indicated. Antibiotic coverage for central venous catheters may be provided at the discretion of the investigator.

5 CORRELATIVE STUDIES FOR RESEARCH

5.1 BIOSPECIMEN COLLECTION

Blood and tissue are tracked at the patient level and can be linked to all protocols on which the patient has been enrolled. Samples will be used to support the specific objectives listed in the treatment protocol(s), e.g., immunologic monitoring, cytokine levels, persistence, as well as to support long-term research efforts within the NCI-SB and with collaborators as specified in protocol 03C0277.

Samples will be ordered in CRIS and tracked through the 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.

Tubes/media may be adjusted at the time of collection based upon materials available or to ensure the best samples are collected for planned analyses.

5.2 Samples Sent to Dr. Figg's Blood Processing Core (BPC)

- Venous blood samples will be collected in either a 4-mL or an 8-mL SST tube to be processed for serum and stored for future research.
    - Record the date and exact time of draw on the tube. Blood tubes may be kept in the refrigerator until pick-up.
    - For sample pick-up, page 102-11964.
    - For immediate help, call 240-760-6180 (main BPC number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).
    - For questions regarding sample processing, contact the BPC at NCIBloodcore@mail.nih.gov or 240-760-6180.
    - The samples will be processed, barcoded, and stored in the BPC until requested by the investigator.

- Plasma preparation for cell free (cf) DNA extraction
  - Collect 10 mL venous blood in EDTA (purple top) collection tube. Record time of blood draw on tube.

  Bring blood tube to lab stat for processing*; page 102-11964 for sample pick-up.

5.3 SAMPLES SENT TO SURGERY BRANCH CELL PRODUCTION FACILITY (SB-CPF)

- Venous blood samples will be collected in 8-mL CPT tubes to be processed and stored for future research.
  - Record the date and exact time of draw on the tube. Blood tubes are kept at room temperature until pick-up.
  - Samples will be transported to the SB-CPF within 24 hours of blood draw.
  - The samples will be processed, barcoded, and stored in the SB-CPF.

5.4 SAMPLE COLLECTION SCHEDULE

Collection Point**	Test/Assay	Volume of Blood (approx.)	Type of Tube	Location of Specimen Analysis
Prior to Preparative Regimen (D-7)	Immune-Monitoring	40 mL 8 mL 4 mL[1]	CPT SST SST	SB-CPF BPC BPC
	Plasma Separation[4]	10 mL	EDTA	BPC
Prior to Cell Infusion	Immune-Monitoring	8 mL[2] (D0)	SST	BPC
	Plasma Separation	10 mL (D-2, D0)	EDTA	BPC
Post-Cell Infusion	Immune-Monitoring	40 mL[3] 8 mL[2,3]	CPT SST	SB-CPF BPC
	Plasma Separation	10 mL (D4, D7, D11, D14)	EDTA	BPC
Post-Treatment Follow-Up)[5]	Immune-Monitoring	8 mL 40 mL	SST[5] CPT	BPC SB-CPF
	Plasma Separation	10 mL	EDTA	BPC

[1] Drawn daily; starting first day of chemotherapy through day of discharge.

[2] For cytokine analysis.

[3] Once total lymphocyte count is > 200/mm^3, samples will be drawn on Monday, Wednesday, and Friday x 5 days, and then weekly (while the patient is hospitalized).

[4] For cell-free DNA extraction.

[5] At each follow-up evaluation (see Section **3.6** for follow-up evaluation schedule). SST tubes are only drawn for the first 3 months after cell infusion.

**Collection times may need to adjusted for holidays and weekends and will be discussed with the PI or proxy.

5.5 IMMUNOLOGICAL TESTING

- Apheresis may be performed prior to and 6 weeks (± 2 weeks) following the administration of the cell product. At other time points, patient PBL will be obtained from whole blood by purification using centrifugation on a Ficoll cushion.
- Samples of all infused cell products will be cryopreserved, and extensive retrospective analysis of infused cell phenotype and function will be performed to attempt to find *in vitro* characteristics of the infused cells which correlate with *in vivo* antitumor activity. Analyses of PBL samples will include evaluation of the activity, specificity, and telomere length of the infused PBL.
- Lymphocytes will be tested directly and following *in vitro* culture using some or all the following tests. Direct immunological monitoring will consist of quantifying T-cells reactive with targets FACS analysis using mouse V-beta antibody. *Ex vivo* immunological assays will consist of cytokine release by bulk PBL (± peptide stimulation) and by other experimental studies such as cytolysis if sufficient cells are available. If cell numbers are limiting, preference will be given to the direct analysis of immunological activity. Immunological assays will be standardized by the inclusion of 1) pre-infusion PBMC and 2) an aliquot of the transduced PBMC cryopreserved at the time of infusion. In general, differences of 2- to 3-fold in these assays are indicative of true biologic differences.

5.6 MONITORING GENE THERAPY TRIALS: PERSISTENCE AND RCR

- Engineered cell survival. TCR and vector presence will be quantitated in PBMC samples using established PCR techniques. Immunological monitoring using both tetramer analysis and staining for the TCR will be used to augment PCR-based analysis. This will provide data to estimate the *in vivo* survival of lymphocytes derived from the infused cells. In addition, measurement of CD4 and CD8 T-cells will be conducted and studies of these T-cell subsets in the circulation will be determined by using specific PCR assays capable of detecting the unique DNA sequence for each retroviral vector engineered T-cell. Note: Samples will be batched and assayed at the conclusion of the study.
- Patients will be co-enrolled on protocol 09C0161 and will adhere to the follow-up schedule described in that protocol. In brief, patients' blood samples will be obtained and undergo analysis for detection of RCR by qPCR prior to cell infusion, and at 3-, 6-, and 12-months post-cell administration. If all post-treatment RCR assays for an individual patient are negative during the first year (i.e., the 3-, 6-, and 12-month timepoints), collection of the yearly follow-up samples will be discontinued for that individual, and yearly review of medical history will be sufficient for that patient. Additionally, a sample of the cell infusion product will be tested by S+L-.
- If a patient has missed one or more of the post-treatment 3-, 6-, and 12-month timepoint sample collections but has at least three consecutive post-administration samples that have all tested negative, then collection of the yearly follow-up samples will be discontinued for that individual and yearly review of medical history will be sufficient for that participant.

- The individual will also undergo an annual physical exam (performed either at NIH or locally) for the first five years of follow-up. If the physical exam is performed locally, records will be obtained by the Surgery Branch. After the Year 5 follow-up is complete, subsequent follow-up for the remainder of the follow-up period (i.e., Years 6-15) will occur by phone and will include a review of the individual's medical history.
- Attempts to meet all post-treatment timepoints in a timely manner will be made. However, due to patients' particular circumstances once they are taken off the treatment protocol, any timepoint may be incomplete, delayed, or even missed. Patients on this study are being treated in coordination with their local physicians for progressive disease and coordination can be difficult. This is to be expected and all attempts to collect complete information will be documented.
- If a patient dies of a suspected retrovirus-associated disease or develops a secondary cancer during this trial, efforts will be made to assay a biopsy sample for RCR. If any post-treatment samples are positive, further analysis of the RCR and more extensive patient follow-up will be undertaken, in consultation with the FDA. The RCR qPCR assay detects the presence of the GaLV envelope gene while the S+L- assay detects the presence of the GaLV envelope protein. Both assays are performed under contract by the Indiana University Gene Therapy Testing Laboratory or other qualified testing facilities. The results of these tests are maintained by the contractor performing the RCR assays and by the NCI-SB research team.

5.7 SAMPLE STORAGE, TRACKING, AND DISPOSITION FOR SB-CPF

Blood and tissue collected during the course of this study will follow the Cell Tracking and Labeling System established by the SB-CPF. The Cell Tracking and Labeling System is designed to unambiguously ensure that patient/data verification is consistent. The patients' cell samples (blood or tissue) are tracked by distinct identification labels that include a unique patient identifier and date of specimen collection. Cryopreserved blood and tissue samples also bear the date the sample was frozen. All cryopreserved samples are tracked for freezer location and storage criteria. All samples are stored in monitored freezers/refrigerators in 3NW NCI-SB laboratories at specified temperatures with alarm systems in place. Serum samples will be sent to the BPC for storage. Samples will be barcoded and stored onsite or offsite at NCI Frederick Central Repository Services in Frederick, MD. All samples collected (blood or tissue) are entered into a central computer database with identification and storage location, and this database is backed up every night.

If, at any time, a patient 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.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be as per the requirements in Section **7.2**.

Blood and tissue collected during the course of this study will be stored, tracked, and disposed of as specified in protocol 03C0277.

5.8 SAMPLE STORAGE, TRACKING, AND DISPOSITION FOR BPC

5.8.1 Sample Data Collection

All samples sent to the BPC will be barcoded, with data entered and stored in the Labmatrix utilized by the BPC, and data will be updated to the NCI-SB central computer database weekly. This is a secure program, with access to Labmatrix limited to defined BPC personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by Dr. Figg. 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 patients without Labmatrix access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the Clinical Center patient 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.).

5.8.2 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 NIH Intramural IRB-approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator (PI) to ensure that the samples requested are being used in a manner consistent with NIH Intramural IRB approval.

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

If, at any time, a patient 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.

Sample barcodes are linked to patient 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 patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be as per the requirements in Section **7.2**.

Blood and tissue collected during the course of this study will be stored, tracked, and disposed of as specified in protocol 03C0277.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The 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 PI, associate investigators (AI), 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.

All adverse events (AEs), including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the first study intervention (the start of cyclophosphamide), through the first follow-up evaluation (6 weeks [± 2 weeks]) following administration of the cell product) or until off-study, whichever comes first. Beyond the first follow-up evaluation, only AEs which are serious and related to the study intervention need to be recorded.

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

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

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

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

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in Section **7.2.1**.

6.1.1 Exclusions to Routine Adverse Event Recording

Patients will be receiving multiple agents, which include commercially available agents (fludarabine, cyclophosphamide, aldesleukin, and supportive medications) in combination with the investigational agent; therefore, grade 1 events not related to the cell product will not be recorded.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

Coded, linked human data generated for use in future and ongoing research will be shared through a NIH-funded or approved repository (ClinicalTrials.gov) and BTRIS. At the completion of data analysis, data will be submitted to ClinicalTrials.gov either before publication or at the time of publication or shortly thereafter. Data may also be used to support long-term research efforts within the NCI-SB, and coded, linked data may also be shared with collaborators as specified in protocol 03C0277.

6.2.2 Genomic Data Sharing Plan

The NIH Genomic Data Sharing Policy does not apply to this study.

6.3 RESPONSE CRITERIA

For the purposes of this study, patients should be re-evaluated for response at 6 and 12 weeks (± 2 weeks), then every 3 months (± 1 month) x3, then every 6 months (± 1 month) x 2 years following administration of the cell product, then as per PI discretion. In addition to a baseline scan, confirmatory scans should also be obtained at least 4 weeks (but not less than 4 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)[26]. 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 v1.1 criteria.

6.3.1 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: ≥ 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.

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. Bone lesions, 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.

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.

6.3.2 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.

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). 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: While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible ‘new’ disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

- Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.

- 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.
- 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.

6.3.3 Response Criteria Definitions

6.3.3.1 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 progression.)

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.

6.3.3.2 Evaluation of Non-Target Lesions

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

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

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or PI).

6.3.3.3 Evaluation of Best Overall Response

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

For Patients 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 weeks confirmation**
CR	Non-CR/Non-PD	No	PR	≥ 4 weeks confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/Not evaluated	No	PR	
SD	Non-CR/Non-PD/Not evaluated	No	SD	Documented at least once ≥ 4 weeks from baseline**
PD	Any	Yes or No	PD	No prior SD, PR, or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST v1.1 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: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration.*" Every effort should be made to document the objective progression even after discontinuation of treatment.

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

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/Non-PD	No	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.

6.3.3.4 Duration of Response

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

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

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

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient 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 website (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 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 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

7.4.1 Serious Adverse Event Reports to IBC

The PI (or delegate) will notify IBC of any unexpected fatal or life-threatening experience associated with the use of anti-KRAS G12D mTCR 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 anti-KRAS G12D mTCR, but are not fatal or life-threatening, much 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 MedWatch Form FDA 3500A or equivalent.

7.4.2 Annual Reports to IBC

Within 60 days after the one-year anniversary of the date on which the IBC approved the initial protocol, and after each subsequent anniversary until the trial is completed, the PI (or delegate) shall submit the information described below. Alternatively, the NIH Intramural 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.

7.4.2.1 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:

- Title and purpose of the trial
- Clinical site
- Principal Investigator
- Clinical protocol identifiers
- Participant population (such as disease indication and general age group, e.g., adult or pediatric)
- 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
- Status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed
- If the trial has been completed, a brief description of any study results.

7.4.2.2 Progress Report and Data Analysis

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

- Narrative or tabular summary showing the most frequent and most serious adverse experiences by body system
- Summary of all serious adverse events submitted during the past year
- 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
- Brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

7.5 NIH Required Data and Safety Monitoring Plan

7.5.1 Principal Investigator/Research Team

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

All data will be collected in a timely manner and reviewed by the PI or AI. Events meeting requirements for expedited reporting as described in Section **7.2.1** will be submitted within the appropriate timelines.

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

7.5.2 Safety Monitoring Committee (SMC)

This protocol will be periodically reviewed by an intramural Safety Monitoring Committee. 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 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 PI 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 patient 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 patient 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 patient 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 patient 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 (21 CFR 312.32).

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 PI 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 PI or sub-investigator.

For timeframe of recording adverse events, please refer to Section **6.1**. All SAEs 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 a protocol-defined serious criteria that requires 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

8.4.1 Disease Progression

Death or hospitalization that is deemed to be due to disease progression, and not attributable to the intervention will not be reported as an SAE. The event, and the assessment that it was caused by disease progression will be documented in the medical records. The causality assessment of Hospitalization will be re-evaluated any time when new information is received. If the causality assessment changes from disease progression to related to the study intervention, an SAE report will be sent to the Sponsor immediately in an expedited manner according to Section **8.3**. If there is any uncertainty whether the intervention is a contributing factor to the event, the event should be reported as an AE or SAE as appropriate.

8.4.2 Known Side Effects

The investigators are requesting a waiver from reporting specific events in an expedited manner to the CCR. Patients will be receiving commercially available agents, such as fludarabine, cyclophosphamide, and aldesleukin. The majority of toxicities observed on NCI-SB ACT protocols are expected toxicities of the non-myeloablative, lymphodepleting preparative regimen or IL-2 and occur in approximately 95% of the patients enrolled; therefore, we are requesting a waiver from reporting the following events in an expedited manner to the CCR.

- Grade 3 or greater myelosuppression, defined as lymphopenia, neutropenia, decreased hemoglobin, and thrombocytopenia.
- Grade 3 or greater nausea, vomiting, mucositis - oral, anorexia, diarrhea, fever, chills, fatigue, and rash maculo-papular.
- Grade 3 hypoxia, dyspnea, hematuria, hypotension, sinus tachycardia, urine output decreased, confusion, infections, and febrile neutropenia.
- Deaths due to progressive disease

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 patients 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 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 (21 CFR 312.32(c)(1)(iv)).

8.5 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

8.5.1 Maternal Exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor 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.

8.5.2 Paternal Exposure

Male patients should refrain from fathering a child or donating sperm from the time of enrollment to the study, during study therapy, and for 120 days after the last dose of study drugs .

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies occurring from the date of the first dose until 120 days 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.

8.6 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. 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.

8.7 SPONSOR PROTOCOL DEVIATION REPORTING

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.

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; and,
- 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 starts 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 Efficacy Endpoint

The primary objective of this trial is to determine whether the combination of high-dose aldesleukin, lymphodepleting chemotherapy, and an infusion of anti-KRAS G12D mTCR-gene engineered lymphocytes is associated with a modest fraction of patients who can experience a clinical response (PR+CR) to therapy. Thus, clinical responses will be the primary efficacy endpoint.

10.2 SAMPLE SIZE DETERMINATION

The study will be conducted using a phase I dose escalation followed by a phase II Simon minimax design in two cohorts: Cohort 2a, patients with RAS G12D pancreatic cancer, and Cohort 2b, patients with RAS G12D non-pancreatic cancer.

Following dose escalation of cells, the objective will be to determine if the combination of high-dose aldesleukin, lymphodepleting chemotherapy, and anti-KRAS G12D mTCR-gene engineered lymphocytes, given at the MTD of cells, is associated with a clinical response rate that can rule out 5% (p0=0.05) in favor of a modest 20% PR+CR rate (p1=0.20) in each of the two cohorts in Phase II.

For each cohort in the Phase II portion of this study, using a two-stage Simon minimax design, with alpha=0.10 (10% probability of accepting a poor therapy) and beta=0.20 (20% probability of rejecting a good therapy), initially 12 evaluable patients will be enrolled. If 0 of the first 12 evaluable patients experience a clinical response, then no further patients will be enrolled in that cohort. If 1 or more of the first 12 evaluable patients enrolled have a clinical response, then accrual will continue until a total of 21 evaluable patients have been enrolled in that cohort. As it may take several weeks to determine if a patient has experienced a clinical response, a temporary pause of up to 6 months in the accrual to the trial may be necessary to ensure that enrollment to the second stage is warranted. If 1-2 of the 21 evaluable patients have a clinical response, then this will be considered inadequate for further investigation. If 3 or more of the 21 evaluable patients have a clinical response, then this will indicate that this strategy provides a new approach that may be worthy of further consideration in that cohort. Under the null hypothesis (10% response rate), the probability of early termination per cohort is 54%.

The dose escalation portion of the study may require up to 6 patients per dose levels 1-7. For purposes of sample size estimation, we will assume that as few as 21 and no more than 24 patients will be required to perform the initial safety evaluation. In order to complete the dose escalation phase and Phase II, a total of up to 24+46 =70 patients may be required (allowing up to 2 inevaluable patients per Phase II cohort). Up to 6 patients enrolled at the MTD will count towards the accrual in the Phase II portion of the trial if they are evaluable for response and if they would be fully eligible for enrollment in the Phase II portion of the trial. Provided that about 2-3 patients per month will be able to be enrolled onto this trial, approximately 2-3 years may be needed to accrue the maximum number of required patients. However, as adequate responses to proceed to the second stage of accrual may not occur, the trial may end up accruing many fewer patients.

10.3 POPULATIONS FOR ANALYSES

10.3.1 Evaluable for Toxicity

All patients will be evaluable for toxicity from the time of their first treatment with cyclophosphamide.

10.3.2 Evaluable for Objective Response

Only those patients 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. These patients will have their response classified according to the definitions stated in Section **6.3.3**.

10.3.3 Evaluable for Non-Target Disease Response

Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one course 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.

10.4 STATISTICAL ANALYSES

10.4.1 General Approach

In the Phase I cohort, the toxicities experienced by patients at each dose level will be reported per dose level. In the two Phase II cohorts, clinical responses will be determined and the fraction with a clinical response will be reported, along with confidence intervals.

10.4.2 Analysis of the Primary Efficacy Endpoint

In the Phase I cohort, the toxicities experienced by patients 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 patients 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.

In the Phase II cohorts, the fraction of patients who experience a clinical response in each cohort will be reported along with 80% and 95% two-sided confidence intervals.

10.4.3 Safety Analyses

Safety will be analyzed by identifying the type, frequency, and severity of any adverse events.

10.4.4 Halting Guidelines and Planned Interim Analyses

Study halting guidelines are as described in Section **3.2**. As noted in Section **10.2**, results after the first stage of a Simon minimax two-stage design in each Phase II cohort will be examined to determine if the minimum number of responses has been obtained.

10.4.5 Sub-Group Analyses

Analyses will be performed separately by cohort in Phase II (RAS G12D pancreatic cancer and RAS G12D non-pancreatic cancers).

10.4.6 Exploratory Analyses

To determine *in vivo* survival of mTCR gene-engineered cells. This will be determined by measuring the number of T-cells in circulation that express mTCRs by fluorescence staining at multiple time points after cell administration.

11 COLLABORATIVE AGREEMENTS

A Cooperative Research and Development Agreement (CRADA #03168) was previously established with Kite Pharma, Inc. Per CRADA #03168, specimens and data were shared with Kite to support their efforts in the development of TCR products. The CRADA was terminated in September 2021 and no specimens or data were shared with Kite after this point.

12 HUMAN SUBJECTS PROTECTIONS

12.1 RATIONALE FOR SUBJECT SELECTION

The patients to be entered in this protocol have unresectable or metastatic cancer which is refractory to standard therapy, and limited life expectancies.

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

Because patients on previous trials of TCR T-cells have experienced hypotension, tachycardia, prolonged fevers, and depressed myocardial function, participation in this trial clearly carries significant risk. In many patients on prior TCR trials, toxicities were severe enough to require intensive care unit admission. We will limit enrollment to patients 72 years of age or less because based on our admittedly limited experience with prior TCR T-cell clinical trials, younger patients tolerate and recover from these toxicities better than elderly patients.

12.2 PARTICIPATION OF CHILDREN

The use of the non-myeloablative, lymphodepleting preparative regimen in this protocol is a major procedure which entails serious discomforts and hazards for the patient, such that fatal complications are possible. It is therefore only appropriate to carry out this experimental procedure in the context of life-threatening metastatic cancer. Since the efficacy of this experimental procedure is unknown, it does not seem reasonable to expose children to this risk without further evidence of benefit. Should results of this study indicate efficacy in treating metastatic KRAS G12D mutated cancer, which is not responsive to other standard forms of therapy, future research can be conducted in the pediatric population to evaluate potential benefit in that patient population.

12.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling on the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (see Section **12.4**), all subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study.

The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation to assess ongoing capacity of the subject and to identify an LAR, as needed. .

Please see Section **12.5.1** for consent procedure.

12.4 Evaluation of Benefits and Risks/Discomforts

All patients in this protocol have incurable metastatic or recurrent/refractory, locally advanced tumors expressing G12D mutated KRAS and limited life expectancies The experimental treatment has a chance to provide clinical benefit though this is unknown. These potential benefits could include shrinking of the tumor(s) or lessening of cancer-related symptoms. The risks in this treatment are detailed in Section **14**. The success of this effort cannot be predicted at this time.

The risks of each procedure/test are listed in the consent form, as well as the implications of the procedure/test results and will be discussed thoroughly with the patient prior to the intervention. When the procedure/test poses any risk to the patient, an additional consent will be obtained, e.g., surgical consent.

12.4.1 Blood Collection

Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting. The most amount of blood to be collected at any one timepoint is about 8 tablespoons.

12.4.2 Cell infusion

Based on our experience giving similar types of cells grown in the laboratory, the following side effects may occur: fever, chills and shortness of breath, which may last for a few hours (common); lung congestion, flu-like symptoms, fluid retention; rarely – severe reaction which could include very low blood pressure and damage to the heart, lungs and/ or kidneys.

12.4.3 Catheter insertion

Insertion of catheters can cause, site pain, inflammation of the vein, bruising, infection, blood clots, leak of the infused liquid, and if this is a central line, puncture of the lung that can result in lung collapse.

12.4.4 Electrocardiogram

Some skin irritation can occur where the ECG/EKG electrodes are placed. The test is completely painless, and generally takes less than a minute to perform.

12.4.5 Urine collection

There are no physical risks associated with urine collection.

12.4.6 Photography

There are no known risks to having photographs taken of skin lesions. Any identifying features, such as the face, will not be included in the photographs.

12.4.7 Leukapheresis

There may be pain and/ or bruising at the IV site. Patients may also experience tingling of the lips and/ or fingers, feel faint or light-headed. Rarely this procedure may cause bleeding or infection at the IV site.

12.4.8 Imaging

X-rays, CT, PET, and/or MRI scans may be used to monitor a patient's disease on this study. CT and PET scans expose a patient 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 patients 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.

MRI scans may also involve the use of gadolinium contrast. Although most of the gadolinium is excreted in urine, FDA recently 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. People with kidney disease are at risk for a serious reaction to gadolinium contrast called “nephrogenic systemic fibrosis” which 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.

12.4.9 Risks from Radiation Exposure

The procedures for performing the 1 chest X-ray, 5 CT scans, and 1 PET scan will follow clinical policies; no special procedures apply to these assessments for research purposes. In summary, the total additional radiation dose for research purposes will be approximately 6.1 rem. Such radiation exposure is associated with an increased risk of cancer.

12.5 CONSENT PROCESS AND DOCUMENTATION

Prior to consideration of this experimental treatment protocol, patients will have already been consented and enrolled on two additional Surgery Branch protocols: 99C0128 for initial screening evaluation and 03C0277 to obtain autologous blood and/or tissue necessary to prepare for this trial. If the patient has a tumor that is found to be KRAS G12D mutated and they are HLA-A*11:01, the patient will be presented with a detailed description of this trial. If the lymphocytes can be generated for infusion and the patient meets the thorough screening for eligibility, when appropriate, the patient, with family members or friends at the request of the patient, will be presented with the informed consent document for this protocol.

The informed consent document will be provided to the patient as a physical or electronic document 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. Manufacturing of the cell product will not begin prior to determination of eligibility, obtaining informed consent, and enrolling the patient 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 patient/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and patient/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the patient/consent designee 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.

Note: 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 permits the use of 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 subject 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.

12.5.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in Section **12.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **12.5**.

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, funding agency and the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. 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

The 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 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 stored at the 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 NIH.

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 INFORMATION

14.1 INVESTIGATIONAL REGIMEN

14.1.1 Anti-KRAS G12D mTCR Transduced PBL

Description: The procedure for expanding the human PBL and the CoA are similar to those approved by the Food and Drug Administration, and used at the NCI in ongoing protocols evaluating cell therapy in the NCI-SB. This product will be provided for investigational use under BB-IND 18470. The CoA for the final infusion product is found in BB-IND 18470. The PBL will be transduced with retroviral supernatant containing the alpha- and beta-chain genes of the anti-KRAS G12D mTCR. PBL will be grown and expanded for this trial according to Standard Operating Procedures which can be found in the NCI-SB BB-MF 13782.

The retroviral vector supernatant (PG13-MSGV8-KRAS G12DmTCR (1) encoding a TCR directed against KRAS G12D, was prepared and preserved following cGMP conditions in the IU-VPF. A murine alpha/beta TCR recognizing mutated KRAS G12D in the context of HLA-A*11:01 was made by vaccinating a mouse transgenic for HLA-A*11:01. Two weeks after completion of vaccination with the VVVGADGVGK peptide mixed with an HBC core helper peptide in incomplete Freund's adjuvant, splenocytes and draining lymph nodes were harvested, plated into 24-well plates at a cell concentration of one million cells/well, and stimulated with the KRAS G12D-derived peptide VVVGADGVGK. Re-stimulation at one week was done and T-cells from reactive cultures were analyzed by RNA extraction, cDNA synthesis, and 5'-RACE to clone TCRs.

Two dominant TCR alpha chains and one dominant beta chain were identified by 5'RACE from KRAS G12D-reactive splenocytes. Retroviral vectors were constructed for each individual chain and screened for correct pairing by co-transducing alpha- and beta-chains into anti-CD3 stimulated HLA-A*11:01 positive PBL. One candidate TCR, TRAV4-4*01 paired with TRBV12-2*01 had specific reactivity against KRAS G12D peptide (pulsed onto COS7/A11), and COS7 transfected with HLA-*A11:01 and KRAS G12D. This alpha/beta TCR pair was synthesized as a single bicistronic vector with the two encoded TCR chains joined with a 2A region linker and cloned into the pMSGV1 retroviral vector. Donor PBL transduced with this retrovirus also recognized HLA-A*11:01+, G12D mutated KRAS+ tumor lines but did not recognize multiple KRAS G12D-negative and HLA-A*11:01 negative human tumors lines. In order to generate a PG13 packaging cell clone, Phoenix-ECO cells were transiently transfected with the MSGV1 transfer vector described above encoding the anti-KRAS G12D mTCR. The transient ecotropic vector is loaded onto retronectin-coated plates which are then used to transduce PG13 cells twice prior to limiting dilution cloning and clone selection. Multiple clones were selected for testing by RNA dot blotting. Retroviral supernatants from these clones were collected and used to transduce the PBL of two HLA-A*11:01+ donors. Efficiency of transduction was evaluated by FACS for murine TCR-beta expression and function evaluated by interferon-gamma release against KRAS G12D+, HLA-A*11:01+ tumors, and transfected cell lines. TCR transduced PBL with all clones produced large amounts of IFN-gamma. Clone #1 was selected to produce the master cell bank and clinical viral supernatant, and transferred to the IU-VPF. The physical titer will be determined by RNA dot blot. The supernatant upon the completion of production and safety testing will be stored at -80°C at Cryonix in Rockville, MD. This storage facility is equipped with around-the-clock temperature monitoring. Upon request, supernatant will be delivered on dry ice to be used in *in vitro* transduction. There will be no re-use of the same unit of supernatant for different patients. Retroviral titer has been shown to be stable after immediate thawing and immediate administration (coating the tissue culture wells previously coated with Retronectin). Handling of the vector should follow the guidelines of Biosafety Level-2 (BSL-2). The specific guidelines for BSL-2 can be viewed at: http://bmbl.od.nih.gov/sect3bsl2.htm.

Note: Penicillin, streptomycin, and gentamycin will not be used in the manufacture of products for patients with documented allergies to these drugs.

Formulation and Preparation: See above for formulation and preparation procedures.

Stability and Storage: Once the transduced cells meet the release criteria noted in the CoA, cells will be released for patient infusion.

Administration Procedures: See Section **3.3.2** for administration procedures.

Toxicities: Based on our experience giving similar types of TCR cells, the following toxicities may be observed: dyspnea, fever, chills, and rash. Rarely, autoimmune reaction and acute cytokine release syndrome will be observed. One patient treated on this study also experienced grade 3 diarrhea with subsequent hospitalization, possibly related to the TCR cells.

14.1.2 Interleukin-2 (Aldesleukin, Proleukin, Recombinant Human Interleukin-2)

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

How Supplied: Interleukin-2 (aldesleukin) will be provided by the NIH Clinical Pharmacy Department from commercial sources.

Formulation/Reconstitution: Please refer to the package insert for additional information.

Storage: Please refer to the package insert for additional information.

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 RIL-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 diluted with 5% dextrose is chemically stable for 48 hours at refrigerated and room temperatures, 2-30°C. As there are no formal stability studies of aldesleukin diluted with HSA, the expiration time will be limited to 4 hours, per pharmacy guidance.

Administration: The dosage will be calculated based on total body weight. The final dilution of aldesleukin will be infused over 15 minutes (± 5 minutes). Aldesleukin will be administered as an inpatient.

Toxicities: Expected toxicities of aldesleukin are listed in the product label and in **Appendix B** and **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**.

14.1.3 Fludarabine

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

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

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

Stability/ Storage: Please refer to the package insert for additional information.

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

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

or idiopathic interstitial pneumonitis. Tumor lysis syndrome has been rarely observed in fludarabine treatment of CLL. Treatment on previous ACT protocols in the NCI-SB have caused persistently low (below 200) CD4 counts, and one patient developed polyneuropathy manifested by vision blindness, and motor and sensory defects.

14.1.4 Cyclophosphamide

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

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

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

Stability: Please refer to the package insert for additional information.

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

Toxicities: Please refer to the FDA-approved package insert for additional information.

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

chemically interact with urotoxic metabolites of cyclophosphamide (acrolein and 4-hydroxycyclophosphamide) to decrease the incidence and severity of hemorrhagic cystitis.

14.2 SUPPORT MEDICATIONS

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

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

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

Storage/ Stability: Please refer to the package insert for additional information.

Administration*:* Dilute to concentrations ≤ 20 mg mesna/mL fluid in D5W or 0.9% NaCl and to be administered intravenously as a continuous infusion. If the patient is obese (BMI > 35), drug dosage will be calculated using practical weight as described in **Appendix D**. Toxicities include nausea, vomiting, and diarrhea.

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

Filgrastim will be obtained commercially by the Clinical Center Pharmacy Department and is supplied in 300 mcg/mL and 480 mcg/1.6 mL vials. Filgrastim should be refrigerated and not allowed to freeze. The product bears the expiration date. The product should not be shaken. It is generally stable for at least 10 months when refrigerated. The appropriate dose is drawn up into a syringe.

Filgrastim will be given as a daily subcutaneous injection. The side effects of filgrastim are skin rash, myalgia and bone pain, an increase of preexisting inflammatory conditions, enlarged spleen with occasional associated low platelet counts, alopecia (with prolonged use), and elevated blood chemistry levels.

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

TMP/SMX DS will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used for the prevention of pneumocystis pneumonia (PCP). The oral dose is 1 tablet PO daily three times a week (MUST be on non-consecutive days), beginning Day 0 or within one week of anticipated lymphopenia, and continuing for at least six months and until the CD4 count is > 200 for two consecutive measures. 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 8-14 days after beginning the standard dose. Neutropenia, a reduction in the number of neutrophils, can also occur.

Dapsone (in G6PD sufficient patient), atovaquone, or pentamidine may be substituted for TMP/SMX-DS in patients with sulfa allergies.

14.2.3.1 Dapsone

Dapsone will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used for the prevention of PCP. The dose is 100 mg by mouth daily, starting on Day 0 (± 7 days) and continuing for at least six months and until the CD4 count is > 200 for two consecutive measures. It is supplied as 25 mg and 100 mg tablets. Dapsone contains a sulfa group, although the cross-reactivity in patients with sulfa allergies is quite low. Dapsone may be considered in patients with mild to moderate sulfa allergies. Dapsone should be avoided in

patients with severe (i.e., a history of anaphylaxis or other equally serious reaction) reactions to sulfa drugs. Additionally, dapsone has been reported to cause hemolytic anemia is patients with G6PD deficiency. It is recommended that patients be tested for G6PD deficiency prior to the initiation of dapsone therapy. Dapsone is generally well tolerated, but may cause a number of hematologic adverse reactions, including increased reticulocyte counts, hemolysis, decreased hemoglobin, methemoglobinemia, agranulocytosis, anemia, and leukopenia. Other rare but serious adverse reactions include bullous exfoliative dermatitis, Stevens-Johnson syndrome, toxic epidermal necrolysis, pancreatitis, interstitial pneumonitis, and pulmonary eosinophilia. For more detailed information about adverse reactions, consult the package insert.

14.2.3.2 Atovaquone

Atovaquone will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used for the prevention of PCP in patients who cannot tolerate or are allergic to sulfamethoxazole/trimethoprim, dapsone, or pentamidine. Atovaquone may be given as a single daily dose of 1500 mg orally or the dose may be split into 750 mg given orally twice daily. Atovaquone will be started on Day 0 (± 7 days) and will continue for at least six months and until the CD4 count is > 200 for two consecutive measures. Atovaquone is supplied as an oral suspension containing 150 mg/mL. Common adverse reactions to atovaquone include: headache, rash, diarrhea, nausea, vomiting, abdominal pain, cough, and fever. Rare but serious adverse reactions include acute renal failure, hepatitis and hepatic failure, angioedema, pancreatitis, and Stevens-Johnson syndrome. For more detailed information about adverse reactions, consult the package insert.

14.2.3.3 Aerosolized Pentamidine

Pentamidine isethionate (inhalation) will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used for the prevention of PCP in patients who cannot tolerate or are allergic to sulfamethoxazole/trimethoprim, dapsone, or atovaquone. Patients with sulfa allergies will receive aerosolized pentamidine 300 mg via nebulizer within one week prior to admission and will continue monthly for at least six months and until the CD4 count is > 200 for two consecutive measures. Pentamidine is supplied in 300 mg vials of lyophilized powder and will be administered via nebulizer. Toxicities reported with the use of pentamidine include metallic taste, coughing, bronchospasm in heavy smokers and asthmatics, increased incidence of spontaneous pneumothorax in patients with previous PCP infection or pneumatoceles, or hypoglycemia.

14.2.4 Herpes Simplex and Varicella Zoster Virus Prophylaxis

14.2.4.1 Valacyclovir (Valtrex)

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

14.2.4.2 Acyclovir

Acyclovir will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prevent the occurrence of herpes virus infections in patients who cannot take oral medications. It is supplied as powder for injection in 500 mg/vials. Reconstitute in 10 mL of sterile water for injection to a concentration of 50 mg/mL. Reconstituted solutions should be used within 12 hours. IV solutions should be diluted to a concentration of 7 mg/mL or less and infused over one hour to avoid renal damage. Reversible renal insufficiency has been reported with IV but not oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs have been reported with higher doses of acyclovir. Should this occur, a dosage adjustment will be made or the drug will be discontinued. Stomach upset, headache or nausea, rash or hives; peripheral edema; pain, elevated liver function tests; and leukopenia, diarrhea, lymphadenopathy, myalgias, visual abnormalities and elevated creatinine have been reported. Hair loss from prolonged use has been reported. Acyclovir will not be used concomitantly with other nucleoside analogs which interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling.

14.2.5 Fluconazole

Fluconazole will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will 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 patients who cannot tolerate the oral preparation, fluconazole comes in 2 mg/mL solution for injection and is prepared according to Clinical Center Pharmacy standard procedures. It should be administered at a maximum IV rate of 200 mg/hour.

14.2.6 Ondansetron Hydrochloride

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

14.2.7 Furosemide

Furosemide will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to enhance urine output during the non-myeloablative, lymphodepleting 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.

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16 APPENDICES

16.1 APPENDIX A: 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.

16.2 APPENDIX B: ADVERSE EVENTS OCCURRING IN ≥ 10% OF PATIENTS TREATED WITH ALDESLEUKIN (N = 525)[1]

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

Body System	% Patients
Metabolic and Nutritional Disorders	
Bilirubinemia	40
Creatinine increase	33
Peripheral edema	28
SGOT increase	23
Weight gain	16
Edema	15
Acidosis	12
Hypomagnesemia	12
Hypocalcemia	11
Alkaline phosphatase increase	10
Nervous	
Confusion	34
Somnolence	22
Anxiety	12
Dizziness	11
Respiratory	
Dyspnea	43
Lung disorder[b]	24
Respiratory disorder[c]	11
Cough increase	11
Rhinitis	10
Skin and Appendages	
Rash	42
Pruritus	24
Exfoliative dermatitis	18
Urogenital	
Oliguria	63

Legend:

[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

16.3 APPENDIX C: EXPECTED IL-2 TOXICITIES AND THEIR MANAGEMENT

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

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

* Unless the toxicity is not reversed within 12 hours.

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

16.4 APPENDIX D: MODIFICATION OF DOSE CALCULATIONS* IN PATIENTS WHOSE BMI IS >35

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

1. BMI determination:

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

2. Calculation of ideal body weight:

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

   Example: Ideal body weight of 5'10" male

   50 + 2.3 (10) = 73 kg

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

   Example: Ideal body weight of 5'3" female

   45.5 + 2.3 (3) = 57 kg

3. Calculation of "practical weight":

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

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

16.5 APPENDIX E: IL-2 TOXICITIES OBSERVED IN PATIENTS TREATED AT THE NIH CLINICAL CENTER

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

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

* Eleven patients are in two protocols.