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1. TITLE OF PROTOCOL: Phase I/II study in WT1-expressing non-small cell lung cancer and mesothelioma, comparing cellular adoptive immunotherapy with polyclonal autologous central memory to naïve $CD8^+$ T cells that have been transduced to express a WT1-specific T-cell receptor

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2. INTRODUCTION

Non-small cell lung cancer (NSCLC) is the leading cause of cancer deaths in the United States and worldwide. Advanced NSCLC (stage III and IV) carries a poor prognosis, and even among stage III patients who are candidates for curative intent treatment, the vast majority still relapse, and ultimately die of their disease[1]. For the majority of patients with stage IV, the mainstay of treatment is traditional chemotherapy, which typically only extends life by a few months. Malignant pleural mesothelioma is an uncommon, aggressive form of thoracic cancer which also carries a dire prognosis, especially if it has reached a non-operable stage [2,3].

However, recent observations that NSCLC, and mesothelioma can respond to antibodies that mediate checkpoint blockade, such as anti-programmed cell death-1 (PD-1) or anti-programmed cell death 1 ligand 1 (anti-PD-L1), demonstrate that NSCLC is an immunologically targetable disease and may respond to other immunotherapeutic strategies as well [4-6]. Wilms' tumor antigen 1 (WT1), a protein involved in regulation of gene expression that can promote the malignant phenotype, is over-expressed in 30% to 80% of NSCLCs (adenocarcinoma and squamous cell subtypes), 100% of mesotheliomas, as well as other malignancies, and has qualities making it an attractive immune target [7]. However, the results of vaccination strategies targeting WT1 have been disappointing, likely as a consequence of central and peripheral tolerance mechanisms impeding the generation of robust cellular responses [8].

Our lab has developed methods to isolate and expand high-avidity $CD8^+$ T-cell clones specific for WT1 from normal donors [9]. In a clinical trial for patients with high-risk leukemias undergoing allogeneic hematopoietic cell transplantation (HCT), 11 patients were infused with WT1-specific $CD8^+$ T-cell clones generated from each patient's matched donor [10]. However, the anti-tumor efficacy was limited in part by variability in the avidity of the clones that could be isolated from each donor. This led us to identify and isolate a high-affinity WT1-specific T-cell receptor (TCR) also known as TCR_{C4} for use in a protocol for treatment of patients with leukemia as well as this protocol. To date, 7 AML patients have been treated with cells expressing the TCR_{C4} after HCT, and preliminary data suggest that this approach is safe, as patients have not experienced significant toxicities, including no

evidence of damage to organs expressing physiologic levels of WT1. This reassuring result occurred despite the long-term persistence of the T cells in most patients [10].

The proposed study is a Phase I/II trial aimed at treating up to 20 patients with advanced NSCLC with stage III or IV disease, and patients with inoperable mesothelioma who have received at least one line of prior systemic therapy. Depending on the initial results in patients with metastatic disease, we will include up to 4 patients with operable stage III NSCLC who will be scheduled to receive T cells as an additional component to their neo-adjuvant therapy, and up to 4 patients with advanced pleural mesothelioma who have unresectable disease and have received at least one line of treatment.

We will assess the safety of this approach, the ability of the cells to persist and localize to tumor mostly in patients who receive T cells in the neo-adjuvant setting, and the potential clinical efficacy. We believe that both an initial tumor regression as well as a long-term clinical response to this treatment will correlate with the persistence of a functional population of antigen-specific cytotoxic T lymphocytes (CTL). Although studies have demonstrated that transferred central memory T cells (T_{CM}) can provide enhanced protective immunity *in vivo* [11], increased persistence and therapeutic activity of transferred murine $CD8^+$ CTLs has also been observed when these cells were derived from the naïve pool and primed in the presence of the γ_c-chain cytokine interleukin-21 (IL-21)[12,13]. Thus, the nature of the T cell that would be the most effective source of cells for adoptive tumor therapy remains controversial. This study is therefore also designed to directly evaluate whether $CD8^+$ T cells derived from the naïve subset and primed in the presence of IL-21 **or** $CD8^+$ T cells derived from primed T cells that have differentiated to the T_{CM} subset and not exposed to IL-21 *in vitro* persist longer, localize better to tumor tissue, or are more effective at eliminating detectable tumor.

3. BACKGROUND

3.A. Prognosis and current treatment options for Advanced Stage NSCLC

NSCLC, which is often diagnosed at an advanced stage, is a prevalent disease with a high mortality rate and is the leading cause of cancer deaths in the United States [14]. Although many patients with Stage IIIA disease are candidates for curative-intent therapy, palliative

treatment goals are usually sought for patients who have an extensive mediastinal tumor burden

Platinum-based doublet chemotherapy regimens have become the gold standard for first-line treatment of advanced stage NSCLC, and multiple studies exploring different dosages and combinations have shown an overall survival benefit measured in months (range 2 to 4 months) compared to best supportive care [15]. However, these agents are also toxic, potentially resulting in debilitating side effects such as fatigue, myelosuppression, peripheral neuropathy, renal insufficiency, and ototoxicity [16]. Using conventional chemotherapy as the only treatment, survival beyond 2 years occurs in less than 20% of cases [17]. Clearly, chemotherapy has reached its plateau of benefit and new therapies for this disease are needed.

The identification of driver mutations in the epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) as the primary oncogenic event in a subset of lung adenocarcinomas has also led to the use of targeted treatments. In patients with EGFR exon 19 deletions and the L858R point mutations [18], response rates of up to 70% can be achieved in patients treated with either erlotinib or gefitinib [19-22]. However, other EGFR mutations (eg, T790M and exon 20 insertion) have been associated with much lower response rates and acquired resistance to tyrosine-kinase inhibitors (TKIs) [22]. Additionally, although EGFR mutations are present in up to 40% of NSCLC detected in Asian populations [23], the incidence of the EGFR mutations in Caucasian populations is limited to 5% to 15% [23,24]. Furthermore, patients with mutations in the K-RAS protein, which have a respective prevalence of 10% and 30% in Asian and Caucasian populations, exhibit activation of signaling pathways downstream from the EGFR, and tend to be resistant to the EGFR TKIs erlotinib and gefitinib [25,26]. The most disappointing aspect of this therapeutic approach remains that even responding patients eventually develop resistance, resulting in short-lived responses with a median progression-free survival of 9 to 14 months [20]. Along similar lines, ALK mutations are observed in approximately 4% of an unselected NSCLC population [27-29]. While ALK-positive patients experience a 65% response rate to crizotinib, an ALK inhibitor, these responses are even more short-lived with a median duration of response of 7.7 months or less [30]. Ceritinib, a second generation ALK-inhibitor, was recently

FDA-approved for patients who have progressed on crizotinib; however, this only offers an additional 7 month duration of response, on average, before the cancer inevitably becomes resistant [31]. Thus, despite recent advances in targeted therapies, the need persists to identify additional treatment options, with more durable responses, for these patients. However, in this trial, patients with a mutation in EGFR or ALK must have demonstrated progression or intolerance to at least one of the corresponding targeted therapies.

Thus far, vaccine strategies in NSCLC have failed to demonstrate clinical benefit in phase III trials [32]. Failure to identify suitable target antigens or to mitigate the immunosuppressive tumor micro-environment in order to allow the generation of an effective immune response has been implicated [33]. However, because the vast majority of tumor antigens are over-expressed self-proteins, the lack of clinical efficacy likely reflects, at least in part, the absence in the peripheral repertoire of autologous tumor-reactive effector cells that possess T-cell receptors with sufficiently high affinities for the tumor antigens [8]. Indeed, immune tolerance mechanisms are in place to eliminate potentially self-reactive cells through the thymic selection process in order to avoid auto-immunity, and T cells with high-affinity TCRs specific for candidate tumor antigens that are non-mutated self-antigens are likely candidates for such negative selection [34,35]. Hence, strategies aimed solely at stimulating endogenous T-cell responses to such antigens will likely have limited efficacy.

Approaches using immune-modulating reagents that block the inhibitory PD-1/PD-L1 axis as a means to dampen the loss of function that can be triggered by the binding of PD-1 on the surface of T cells with its ligand PD-L1 expressed on tumor cells have appeared promising [36]. Blocking the PD-1/PD-L1 axis has been shown in early clinical trials to result in durable responses in a fraction of patients with melanoma, renal cell carcinoma, and NSCLC [6,37]. The phase I clinical trials of the anti-PD-1 (nivolumab, BMS-936558) and anti-PD-L1 (BMS-936559) antibodies demonstrated sustained tumor regressions in heavily pretreated patients with advanced NSCLC [4,6]. In the anti-PD-1 trial of 296 patients, 122 patients had NSCLC, 76 of whom were evaluable for efficacy, and confirmed responses were observed in 17% of patients [38] In a comparably designed phase I study employing anti-PD-L1 antibody, 207 patients were enrolled, 75 patients had NSCLC, 49 were evaluable and responses were documented in five patients (10%) [4]. Since then, additional anti-PD-1 and

anti-PD-L1 agents (MK-3475, MPDL3280A, MEDI4736) have shown equally promising results with overall response rates ranging from 15% to 26% in NSCLC [39-43]. Although the majority of patients have not responded to monotherapy with anti-PD-1/PD-L1 blocking agents, these results are still very encouraging with regard for harnessing the immune system. As durable responses have been observed in NSCLC, albeit in a very limited fraction of patients, the results provide evidence that these tumors can be sensitive to a more robust (or less inhibited) T-cell mediated anti-tumor immunity.

In spite of the recent advances, the prognosis of advanced NSCLC remains dismal as the vast majority of patients experience relapse and tumor progression, and ultimately die of their disease after several lines of therapy [1]. Thus, in light of the fact that NSCLC can be responsive to immunomodulation, the development of strategies for targeting tumor-associated antigens that bypass the patient's tolerance mechanisms may provide a potentially effective therapeutic approach. Establishing an antitumor response derived from transfer of adoptively transferred autologous T cells genetically modified to express a high-affinity TCR that recognizes an antigen expressed by NSCLC cells represents one such strategy. Because there are no cross-reactive toxicities, this strategy can be easily combined with other modalities such as chemotherapy and radiation therapy, and can potentially also be combined with PD-1/PD-L1 inhibitors to enhance and sustain responses in NSCLC.

3.B. Rationale for Treating Advanced Stage NSCLC with WT1-specific CD8$^+$ T Cells

Adoptive T-cell therapy with CD8$^+$ CTL specific for antigens expressed by the tumor cells but absent or expressed at only low levels by normal tissue has the potential to selectively eliminate NSCLC cells. WT1, located at 11p13q, encodes a 52-54 kDa protein transcription factor containing four DNA-binding zinc fingers at the C-terminus [44]. *In vitro* and *in vivo* studies in mice have shown that WT1 has multiple functions, including gene transactivation, gene repression, and RNA binding [45]. It is involved in the regulation of genes coding for growth factors (eg, TGFβ, CSF-1), growth factor receptors (eg, insulin-R, IGF-1R, EGFR), transcription factors (eg, EGR, WT1, c-Myc, Pax2, Dax-1, and Sry), and anti-apoptotic molecules (bcl-2 and bcl-xl) [44,46,47]. During embryogenesis, WT1 plays a critical role in the development of the genitourinary tract, spleen, and mesothelial structures [48], but WT1

expression after birth is limited to very low levels predominantly in kidney podocytes, sertoli cells of the testes, granulosa cells of the ovary, mesothelial cells of the lung, the uterus and fallopian tubes, and $CD34^+$ hematopoietic progenitor cells [48-51].

The WT1 gene was originally identified as a tumor suppressor gene in pediatric kidney cancers, but subsequently it became evident that WT1 has a broader role in the oncogenesis of both hematological malignancies and solid tumors. Over-expression of the WT1 gene is detected in many leukemias, including approximately 90% to 100% of cases of acute myeloid leukemia (AML); myelodysplastic syndrome (MDS) and chronic myeloid leukemia (CML); solid tumors including breast, ovarian, and pancreatic cancers; mesothelioma; and in more than 96% of NSCLC by reverse transcription polymerase chain reaction (RT-PCR) [52,53]. Up-regulation of WT1-expression in NSCLC is also evident by immunohistochemistry (IHC) in comparison to adjacent tissues, with WT1 protein demonstrated, depending on the detection antibody used, in up to 72% of NSCLC patients by IHC (**Figure 1**) [54,55]. Contrary to the nuclear staining observed in hematological malignancies and mesotheliomas, WT1 protein is predominantly localized to the cytoplasm of lung cancers [53,56], likely due to shuttling of this mostly intranuclear protein [57].

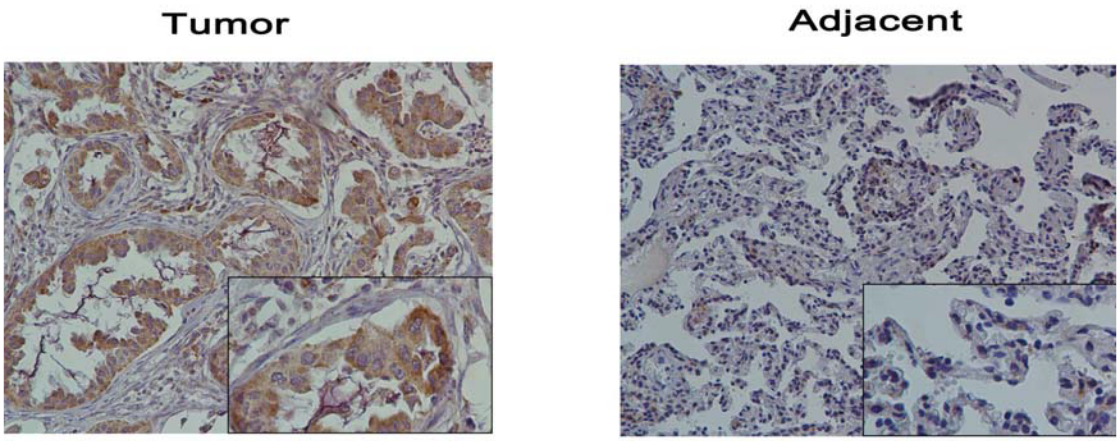


Figure 1. Expression of WT1 assessed with the 6F-H2 antibody clone in NSCLC (left) and adjacent lung tissue (right) [54]

High levels of WT1 expression have been correlated with a poor prognosis in patients with leukemias and breast cancer [58,59]. *In vitro* studies have demonstrated that over-expression of WT1 in NSCLC cell lines enhances viability and proliferation, whereas down-regulation of WT1 by antisense oligonucleotides inhibits the growth of the cell lines [54]. WT1

overexpression also accelerates tumor-cell entry into S-phase by up-regulating Cyclin D1 and phosphorylating and inhibiting retinoblastoma protein (pRB), which promotes NSCLC tumor growth. Furthermore, expression of WT1 is associated with enhanced invasiveness of WT1-expressing NSCLC tumor lines via negative regulation of E-cadherin following direct binding to its promoter [60]. Because WT1 promotes proliferation and oncogenicity, loss of expression is disadvantageous for the tumor, reducing the risk of outgrowth of antigen-loss variants [61]. Sequencing of WT1 genomic DNA in *de novo* NSCLC detected no mutations in the tumor despite the highly unstable genome in NSCLC, further suggesting that loss of WT1 is disadvantageous for the tumor [62].

For this study, we plan to assess NSCLC WT1 expression for each individual patient by staining stored paraffin-embedded tissue obtained at the time the patient is diagnosed or from a subsequent biopsy with the immuno-histochemical method recommended by the WT1 Conference on Harmonization of WT1 IHC using the 6F-H2 mAb (Dako). Using this method 30% to 35% of NSCLC cases stain for cytoplasmic WT1, and it is the only reliable and reproducible method available to date[55,56].

This protocol will examine the activity of autologous T cells that are rendered reactive with WT1 by introduction of a high-affinity WT1-specific TCR (TCR_{C4}), isolated after screening clones from more than 70 normal donors, that recognizes the $WT1_{126\text{-}134}$ epitope (RMFPNAPYL) in the context of the class I major histocompatibility complex (MHC) HLA-A*0201 molecule. This epitope is highly conserved among species and expressed in all described WT1 isoforms, and, as no coding mutations of this epitope have been reported, it is considered a promising immunotherapeutic target [63].

3.C. Prognosis and Current Treatment Options for Advanced Stage Mesothelioma and Rationale for Treating Patients with Mesothelioma with WT1-specific $CD8^+$ T Cells

Malignant pleural mesothelioma is an uncommon, aggressive form of thoracic cancer arising from the mesothelial surfaces of the pleural cavity, and often associated with asbestos exposure. It carries a poor prognosis of 4 to 13 months for untreated patients [64] and 6 to 18 months for treated patients[2,3]. Cure is rare; most patients have advanced disease on presentation, but even operable patients typically relapse despite aggressive trimodality

therapy with surgery, chemotherapy, and radiation. For inoperable pleural mesothelioma, cisplatin in combination with pemetrexed is currently the only FDA-approved regimen, and has demonstrated an increased survival compared to single-agent cisplatin of 12.1 months versus 9.3 months [65]. After progression on first-line chemotherapy, the only available options are off-label uses of other chemotherapy agents, which have largely been found to be ineffective against this cancer [66].

In a recent phase II, single-arm, open-label trial, 29 patients with advanced chemotherapy-refractory mesothelioma were treated with a median of two doses of tremelimumab, an antibody against CTLA-4. There were two partial responses, the longest lasting 18 months, and a 31% disease control rate with a median PFS of 6.2 months. Although the effects were modest, these findings provide evidence that in some cases, mesothelioma can be recognized and controlled by a patients' own T-cell responses once released by checkpoint blockade. Due to the low prevalence of mesothelioma, there is little incentive from the scientific community at large to spend significant time and resources towards developing novel therapies, even though existing treatment options are severely limited and novel therapies are needed for mesothelioma.

Mesothelioma is histologically identified by its expression of WT-1 and thus could be a potential target for WT-1 specific T-cell therapy. Therefore, due to the immunologic feasibility suggested by early response to checkpoint blockade, and the potential for a large impact on a disease with few treatment options, one of the aims of this trial will be to investigate in a preliminary, exploratory manner, the safety of WT-1 TCR cells in a small subset of four patients with mesothelioma.

3.D. Rationale for Using Autologous CD8$^+$ T Cells Transduced with a Lentiviral Vector to Express a High-affinity WT1-specific TCR for Patients with Advanced Stage NSCLC and Mesothelioma

Adoptive transfer of donor-derived *ex vivo*-expanded WT1-specific CD8$^+$ CTLs in patients with NSCLC can potentially bypass the limitations encountered during vaccination by increasing the number and quality of T cells targeting this tumor-associated antigen. Our lab has developed methods to generate high-avidity CD8$^+$ T-cell responses specific for WT1 by

primary *in vitro* sensitization of naïve T cells (T_N) from healthy donors[9], and we completed a clinical trial in allogeneic HCT patients with relapsed leukemia in which WT1-specific $CD8^+$ T-cell clones were generated and expanded from each patient's normal matched donor for use in therapy[10]. Although we successfully generated WT1-specific $CD8^+$ T-cell clones from more than 85% of donors, the avidity of the T cells generated from each donor for the WT1 epitope presented in MHC class I was variable, and the lower avidity responses exhibited less reactivity *in vitro*, which may have limited *in vivo* anti-tumor activity.

Isolating and transferring a high-affinity TCR into primary T cells has the potential to impart specificity as well as high avidity for a cell expressing the desired target antigen. Genes encoding the α and β chains of a TCR have already been introduced into T cells and clinically evaluated in patients [67], and T cells transduced with a MART-1- or gp100-specific TCR were shown in patients with metastatic melanoma to migrate to the site of disease and mediate cancer regression [67,68].

One potential limitation of TCR-transduced T cells is that the introduced TCR chains can inappropriately pair with the endogenous TCR chains, resulting in creation of TCRs with unpredicted and potentially auto-reactive specificities, as well as reducing expression of the correctly paired chains that will decrease the avidity compared to the original "donor" T cell. However, our lab demonstrated that introducing a point mutation into each of the TCR chains that inserts a cysteine (cys) into the Cα and the Cβ domains to promote creation of an interchain disulfide-bond results in preferential pairing of the introduced α and β chains with each other and reduced mismatching with endogenous TCR chains, and enhanced expression compared to the endogenous TCR chains, resulting in the transduced T cells demonstrating higher avidity than cells transduced to express chains without the mutation.[69]

Another modification that has proven beneficial for increasing TCR transgene expression is codon optimization. Redundancy in the genetic code allows some amino acids to be encoded by more than one codon, but certain codons are less optimal for translation than others, due in part to the relative availability of matching transfer RNA (tRNA) [70]. Modifying the TCRα and β gene sequences to encode each amino acid by the optimal codon for

human gene expression, as well as eliminating mRNA instability motifs and cryptic splice sites, has significantly enhanced TCRα and β gene expression.[71]

The WT1-specific TCR being used in this protocol (TCR_{C4}) was selected after screening more than 1 000 T-cell clones isolated from the repertoires of more than 70 normal healthy individuals for avidity, as reflected by increased WT1-specific cytolytic activity for targets expressing decreasing levels of WT1 and by affinity for peptide/MHC tetramers.

To minimize the potential risk of on-target off-tissue toxicity, the selected TCR_{C4} was purposefully isolated from the peripheral repertoire of a healthy HLA*0201^{+} donor, guaranteeing that the TCR had undergone negative selection during thymic development, and that the T cells expressing the TCR were not mediating an autoimmune process in the periphery. The selected TCR was inserted into a third-generation lentiviral construct for expression in human cells and codon-optimized to achieve high-level protein expression. The final construct was engineered to be encoded as a single open reading frame consisting of the TCRβ and TCRα chains separated by a 2A element from the porcine teschovirus (P2A) to ensure coordinated gene expression [72,73], and incorporated complementary cysteine residues following introduction of point mutations in the constant domains of the TCRα and TCRβ genes to promote appropriate inter-chain pairing of the introduced TCR chains [69].

3.E. Rationale for Employing a Third-Generation Lentiviral Vector to Transduce Autologous Well-Differentiated CD8 T Cells

The theoretic risk of insertional mutagenesis with retroviral vectors became realized during two X-linked severe combined immunodeficiency (SCID) gene therapy trials in which five patients developed acute lymphoblastic leukemia (ALL), shown to reflect transactivation of either the LMO2 or CCND2 gene by the retroviral insert in transduced CD34^{+} hematopoietic stem cells (HSCs). This highlighted the propensity of gamma-retroviral vectors (γ-RV) to preferentially insert near genes that are being actively transcribed, which may be particularly problematic in transduced HSCs that express genes that confer self-renewal capacity and a proliferative/survival advantage and are then transplanted into a setting in which they are driven to extensively expand [74].

By contrast, the risks of insertional oncogenesis or other vector-related cellular toxicities are extremely low when targeting peripheral blood T cells, with no AEs reported to date in any clinical trial [68,75-78]. In a recent report of long-term results from the combined data of three clinical trials to evaluate γ-RV-engineered T cells for patients with HIV, insertional oncogenesis was not observed [78]. The transduced cells persisted long term, in some cases more than 10 years, and clinical monitoring of the patients at yearly intervals for cumulatively more than 540 patient-years of observation has not detected evidence of retroviral genotoxicity. In more recent trials with less extensive retrospective data, no toxicities attributable to the administration of T cells transduced to express HLA*0201-restricted MART1 or gp100-specific TCRs using the MSGV1-based RV were observed in 33 patients with metastatic melanoma. The MSGV1-based RV uses the same promoter from the LTR of MSCV that will be used in the lentiviral vector in our trial [68,75]. The MSGV1-based γ-RV was also used to transduce autologous T cells to express an NY-ESO-1-specific TCR, and was infused in 17 patients with metastatic synovial sarcoma or melanoma[77] and in 3 patients to express a carcinoembryonic antigen (CEA)-specific TCR. Again, no insertional mutagenesis events were identified [76].

The risk of insertional oncogenesis can be further decreased with the use of a third-generation self-inactivating (SIN) lentivirus, in which the promoter regions of the long terminal repeats (LTRs) have been truncated and the insert, such as a TCR gene, is expressed under control of an internal murine stem cell virus (MSCV)-based promoter [79]. Transcriptionally active enhancer/promoter elements may influence expression of cellular genes at a distance from the insertion site, independent of the vector type (RV vs lentiviral vector [LV]) and design (LTR-based or SIN) [80]. However, γ-RVs have a predilection toward integrating in the immediate proximity of transcription start sites and deoxyribonuclease I (DNAse I) hypersensitivity sites [81], increasing the probability that the viral LTR transcriptional enhancer will interfere with gene regulation and potentially activate cancer-causing genes [82]. In contrast, LVs are more likely to integrate further away from the transcription start sites into active transcription units and are thus less likely to induce transcriptional activation [83]. Moreover, *in vivo* genotoxicity assays based on the transplantation of transduced tumor-prone *Cdkn2a-/-* murine hematopoietic progenitor cells have directly compared the effect of promoter location within γ-RVs and SIN lentiviral

constructs on the oncogenic potential. Placing the strong LTR-based spleen focus-forming virus promoter as an internal promoter in a construct containing a SIN LTR rather than as a component of the LTR further reduced the propensity for insertional mutagenesis and lymphoid tumors [84].

Despite the risk of insertional mutagenesis being extremely low, it remains justified to initially examine safety, toxicity, and potential efficacy of therapy with transduced T cells in patients with advanced NSCLC, as described in our plan, rather than in patients with better disease prognosis. The potential toxicities are listed in the protocol consent form, and will be discussed with patients as a part of the consent process. Eligible patients who elect to enroll on the protocol will be monitored and managed for potential toxicities as outlined in **Section 15** and **Section 16**, and stopping rules will be applied as described in **Section 21.A.**.

3.F. Rationale for Transducing Autologous Cell Populations Derived from Long-lived Memory and Naïve Cells to Express the High-affinity WT1-specific TCR

The establishment of a persistent functional population of antigen-specific CTL *in vivo* after transfer will likely be necessary to eliminate detectable NSCLC tumor as well as prevent tumor recurrences. Previous T-cell therapy trials have often been limited by the inability of transferred T cells to expand and persist *in vivo* after transfer. The *in vivo* fate of transferred T cells is dependent in part on the intrinsic properties of the T cells from which the infused cells were derived[11,85], and TCR gene therapy provides a unique opportunity to choose the nature and origin of the T-cell type used for adoptive cell therapy.

Conventional $CD8^+$ T cells can be divided into naïve T cells (T_N), and antigen-experienced memory T cells (T_M). Memory T cells can be further divided into T_{CM} and effector memory T cell (T_{EM}) subsets, which have distinct transcriptional programs that dictate many characteristics, including homing, phenotype, and function [86]. When T_{EM} cells are stimulated *in vitro*, they differentiate largely into short-lived effector cells, which effectively kill targets, but generally have limited proliferative capacity and fail to persist for long periods after transfer *in vivo* [85-88].

Studies of transferred purified $CD8^+$ T-cell subsets in a murine lymphocytic choriomeningitis virus (LCMV) infection model revealed that transferred T_{CM} provide enhanced protective immunity from *in vivo* challenge compared to T_{EM} [11]. Although T_{CM} cells expand and also differentiate into effector cells in response to *in vitro* stimulation, studies in non-human primates in which such T_{CM}–derived effectors were transferred revealed that these effector cells have been imprinted and retain some of the beneficial properties of the parent T_{CM} cell from which they were derived, in particular the capacity for self-renewal, which translates into improved *in vivo* persistence and response to antigen challenge *in vivo* [85].

Increased persistence has also been observed with murine $CD8^+$ CTLs derived from the naïve pool when these cells were primed in the presence of the γ_c-chain cytokine IL-21[12,13]. In both murine and human studies, adding IL-21 to T-cell cultures supplemented with Interleukin-15 (IL-15) during the *in vitro* priming of antigen-specific $CD8^+$ T cells has been shown to not only induce greater expansion and prevent apoptosis of the cells responding to antigen stimulation, but to also lead to *in vitro* generation of $CD8^+$ T cells that are $CD28^{hi}$, which reflects a less terminally differentiated phenotype[13,89]. We have also shown in humans that WT1-reactive CTL clones derived from naïve donors and initially primed in the presence of IL-21 expressed significantly higher levels of CD27, CD28, and CD127 prior to infusions, which is again consistent with a less differentiated phenotype [10]. Following infusion, these CTL clones demonstrated enhanced capacities for *in vivo* persistence and proliferation compared to CTL clones generated in the absence of IL-21. These preliminary results in a limited number of patients suggest that CTL clones derived from a naïve subset and primed *in vitro* in the presence of IL-21 may provide an alternative to generating CTL populations for transfer from T_{CM}. However, the most adequate cell subset for transduction and adoptive transfer in a solid tumor setting remains undetermined.

This study is designed to resolve this controversy and evaluate whether <u>$CD8^+$ T cells derived from the T_N and primed in the presence of IL-21</u> **or** <u>$CD8^+$ T cells derived from an already primed T_{CM} subset and not exposed to IL-21</u> *in vitro* persist longer, localize better to tumor tissue, or are more effective at eliminating detectable tumor. Patients enrolled in this trial will receive sorted $CD8^+$ T cells from the T_N subset and the T_{CM} subset that will be subsequently transduced to express the TCR_{C4}. Cells derived from the T_N subset will be

additionally exposed to IL-21 at the time they are stimulated. Prior to transfer, the endogenous TCR usage of both population subsets will be determined by high throughput TCRβ sequencing (HTTCS) [90-92]. This is comparable to 'bar coding' the T_N and T_{CM} populations [93]. The trial will evaluate and compare the behavior of these two T-cell populations after they are infused *in vivo* within the same patients by performing HTTCS on blood and tumor samples and determining respective frequencies, persistence, phenotype, function, and—in a subset of patients treated in the neo-adjuvant setting and patients who can donate tumor tissue (**see Section 15.F.2**)—the localization to tumor. These results will be correlated with the clinical outcomes of the patients.

3.G. Safety Concerns

3.G.1 Pre-clinical murine model for targeting WT1

Expression of WT1 can be detected at low levels in normal kidneys, testes, ovaries, pleura, pericardium, the uterus and fallopian tubes, and $CD34^+$ hematopoietic progenitor cells [51]. Thus, targeting cells that express WT1 raises the concern of on-target/off-tissue toxicities. As WT1 expression in normal and malignant tissues is comparable between mice and humans, we have in part addressed these concerns by developing a mouse model in which on-target toxicities can be assessed using the highest affinity murine WT1-specific TCR that we could isolate from the normal repertoire as well as variants of this TCR that we have mutated to achieve an affinity approximately 200 to 500 fold higher than can be isolated from the normal repertoire. Mice infused with $CD8^+$ T cells isolated from the peripheral pool and transduced with either the naturally isolated or mutated high-affinity TCRs did not demonstrate any toxicity in WT1-expressing organs, and the transduced T cells functioned normally *in vivo*, responding to immunization with a recombinant vector expressing WT1 and recognizing $WT1^+$ tumor cells with no evidence of activation from recognition of normal cells [94].

The safety of T cells transduced to express the enhanced-affinity TCRs raised the question of why these TCRs could not be detected in the normal repertoire. Therefore, we transduced these TCRs into HSC and examined the development and maturation of T cells in the thymus. We found that, unlike those expressing the naturally isolated TCR, developing T cells expressing the enhanced affinity TCRs do indeed undergo negative

selection in the thymus. A fraction of these cells do survive negative selection and emigrate from the thymus, but exhibit down-regulation of the TCR or CD8 or both, resulting in peripheral T with lower avidity than T cells that developed expressing the naturally isolated TCR. These results suggest that negative selection in the thymus is overprotective for WT1-reactive T cells, and thus the risk of on-target/off-tissue toxicities to WT1-expressing organs after infusion of T cells transduced with a WT1-specific TCR derived from a naturally occurring T-cell clone should be low.

3.G.2 Previous Clinical Experience: Targeting WT1 with transfer of donor-derived CD8$^+$ T-cell clones into patients after HCT

Patients who were treated in our previous trial with escalating doses of donor-derived WT1-specific CD8$^+$ T-cell clones showed no evidence of hematopoietic suppression or graft failure, renal failure, pleuritis, pericarditis, abdominal pain (from splenic capsule or ovaries), or testicular or ovarian pain, which could represent damage to organs expressing physiologic levels of WT1 [10]. The "parental" WT1-specific CD8$^+$ T-cell clone C4, from which the TCR to be used in this trial was derived, was isolated from a healthy donor expressing HLA A*0201. This TCR was chosen to maximize the potential for therapeutic activity and minimize the potential risk of on-target/off-tissue toxicity, as the TCR_{C4} represents the *highest-affinity* TCR that we identified from the naïve peripheral repertoire of more than 70 HLA*0201$^+$ donors. Thus, the T cell expressing this TCR had undergone negative selection during thymic development, and, after export from the thymus, remained naïve and was not activated in the periphery.

The **functional avidity of CD8 T cells, as defined by the ability to recognize and lyse target cells expressing limiting amounts of cognate antigen *in vitro*,** is influenced by the multi-parametric binding of the effector- and target-cell populations. This is determined in large part by the affinity of the expressed TCR, although expression of co-stimulatory and adhesion molecules and their ligands, as well as the capacity to express cytolytic molecules, make substantive contributions. The functional avidity of the parental C4 clone, as well as cell products transduced with the TCR_{C4}, were assessed by lysis of TAP-deficient B lymphoblastoid cell line (T2 B-LCL) cells pulsed with decreasing concentrations of $WT1_{126-134}$ peptide. The TCR_{C4}-transduced CD8 T cells exhibited a similar functional avidity both to the best WT1-specific CTL clones infused into patients in our previous study [10] and to the

parental C4 clone from which the TCR was derived (**Figure 2**). Of note, a subset of the clones used in therapy with similar high avidities persisted long-term (> 100 days) without toxicity.

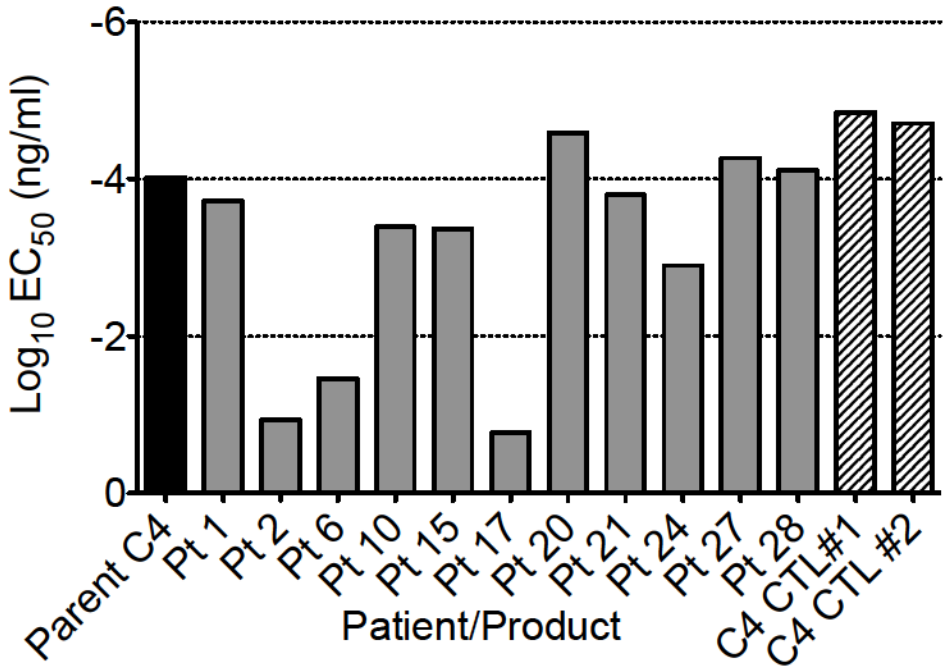


Figure 2. Avidity of products to WT1-pulsed T2 B-LCL

3.H. Previous/Ongoing Human Experience with TCR_{C4}

TCR_{C4} is currently being employed in the clinic to transduce T cells for therapy of patients with high-risk leukemias who have undergone allogeneic HCT and have relapsed or are at a high risk of disease recurrence [95] (**protocol FHCRC #2498**), in a phase I/II trial designed primarily to evaluate the safety and efficacy of intra-patient escalating doses. For the trial, this characterized TCR, specific for the $WT1_{126\text{-}134}$ epitope (RMFPNAPYL), is being introduced into HLA-A*0201-restricted Epstein–Barr virus (EBV)- or cytomegalovirus (CMV)-specific donor cells.

The use of virus-specific (preferably EBV or if necessary CMV) cells for expressing the characterized WT1-specific TCR in the post-HCT setting serves three purposes: 1) restricts the antigen specificity of the endogenous TCRs expressed by the substrate cells to a known foreign pathogen, decreasing the likelihood of transducing donor-derived T cells that recognize host antigens; 2) removes the possibility of transducing T_N cells that contribute

disproportionally to GVHD following HCT [96,97]; and 3) ensures the transduced T cells will contain a large fraction of T cells derived from the T_{CM} cell pool, which should be endowed with properties that enhance *in vivo* persistence after transfer.

3.H.1 FHCRC #2498 Study Schedule

Initially, each patient enrolled on FHCRC protocol #2498 was planned to receive a total of 4 infusions following a dose-escalation schedule: 1×10^9 cells/m^2, 3.3×10^9 cells/m^2, 1×10^{10} and 1×10^{10} cells/m^2 followed by low-dose subcutaneous (s.c.) Interleukin-2 (IL-2) at 2.5×10^5 IU BID × 14 days, administered to enhance the survival of transferred T cells [98] (**Figure 3**). The trial started out as a two-armed study with patients who had no detectable disease after HCT treated on the 'Prophylactic Arm' (Arm 1). For safety concerns, the first 3 to 6 patients (Arm 1/Stage 1) were scheduled to receive infusions separated by a 28-day interval between infusions. If no unexpected toxicities were detected, the interval between infusions was planned to be reduced to 14 days (Arm 1/Stage 2). Patients with relapsed disease after HCT were scheduled to receive infusions on the 'Treatment Arm (Arm 2). The first 3 to 6 patients (Arm 2/Stage 1) were scheduled to receive the same infusion doses as Arm 1/Stage 1, but these were to be separated by a shorter interval to reach higher and potentially therapeutic doses in a limited time. Again, if no toxicities were observed, subsequent patients were to begin with a higher dose of cells (3.3×10^9 cells/m^2), separated by a 14-day interval. Because the dose-escalation schedule was well tolerated and no severe and unexpected toxicities were observed in the first 7 patients treated with up to 10^{10} cells/m^2 transduced with the TCR_{C4}, both the FDA and the FHCRC IRB approved discontinuation of the dose-escalating schedule in May 2014 such that all patients prospectively enrolled (irrespective of their disease burden post-transplant) now receive a first dose of 10^{10} cells/m^2 on day 0, and then a second infusion of 10^{10} cells/m^2 on day 14, this time followed by 14 days of s.c. low-dose IL-2 as soon as the cellular products are available (**Figure 4**).

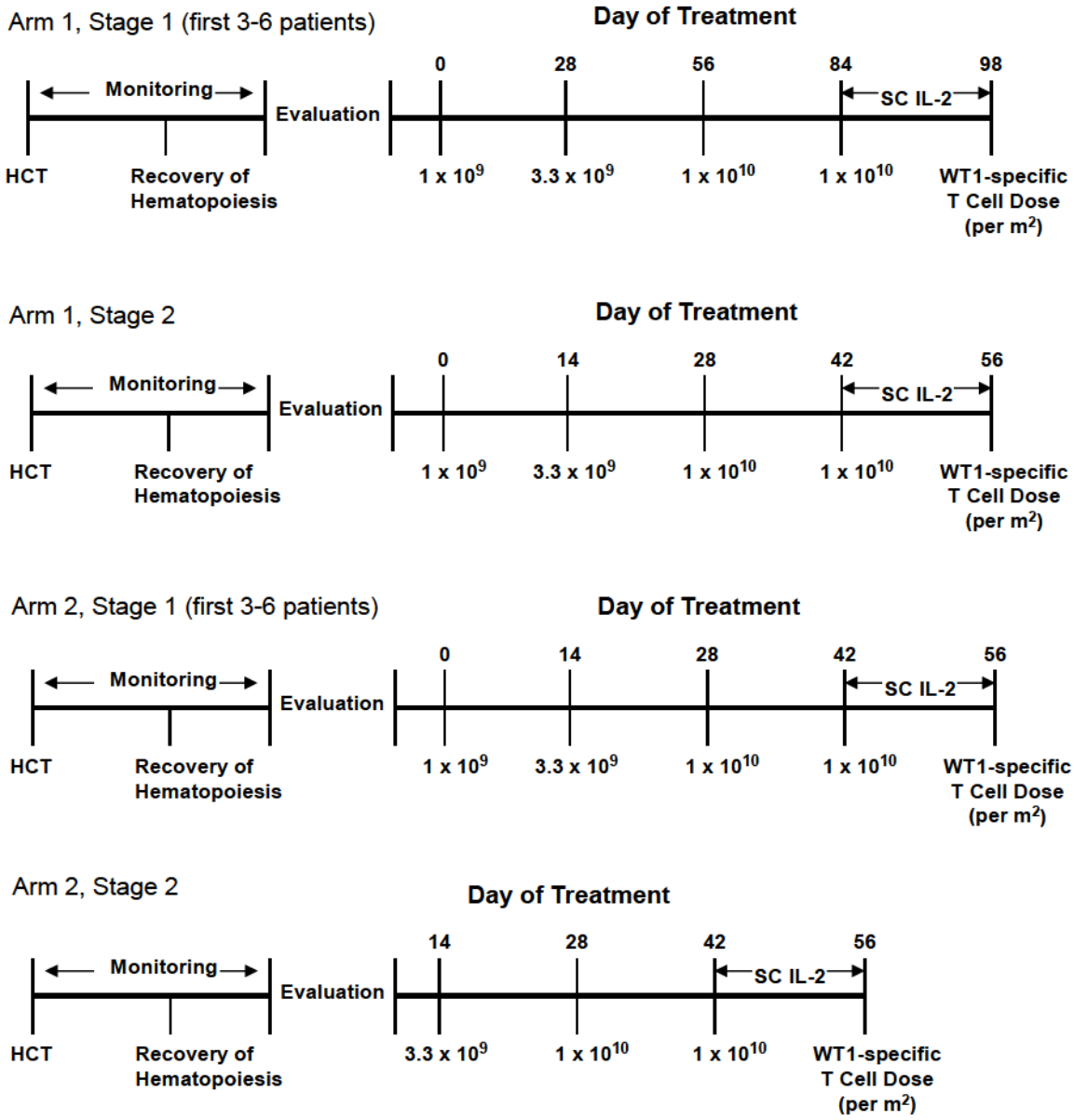


Figure 3. Initial treatment plan of protocol 2498

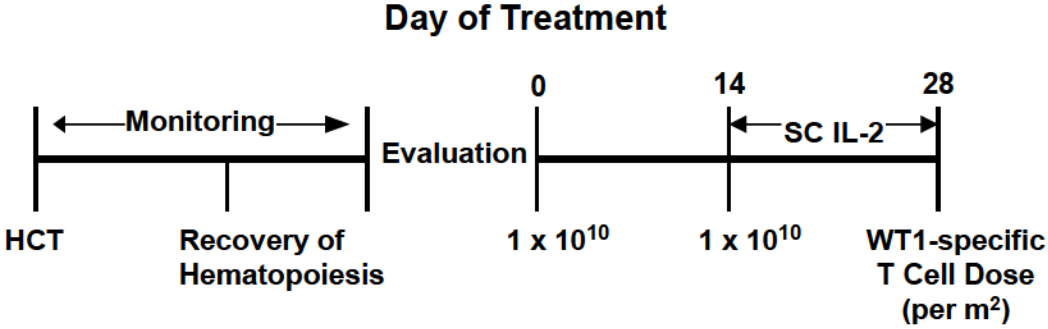


Figure 4. Current treatment plan of protocol 2498

3.H.2 Patient characteristics and persistence of transferred TCR_{C4}-transduced cells on protocol 2498

As of Janary 20th 2015, 16 patients with high-risk AML received a total of 34 escalating doses of donor-derived virus-specific cells (**Table 1**). Ten patients were treated on Arm 2 due to detectable disease post-HCT and 6 patients received prophylactic treatment on Arm 1.

Table 1. Patient characteristics on protocol 2498

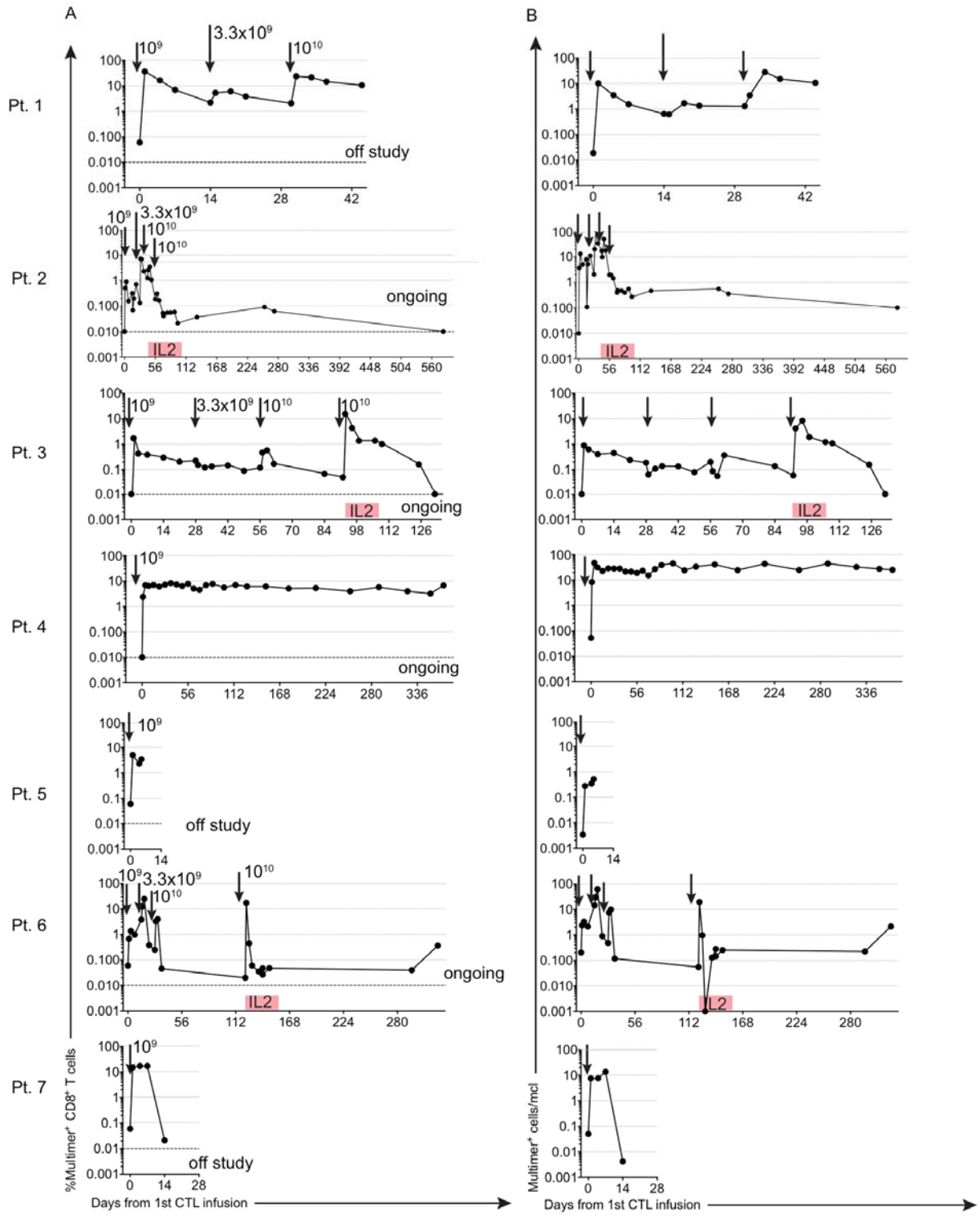
Definitions: M = male; F = female; AML = acute myeloid leukemia; y = years; HCT = hematopoietic cell transplantation; CR = complete response; PB = peripheral blood; MRD= minimal residual disease

Pt. No.	M/F	Age	Disease	2498 Arm	Comments
1	M	56	AML, relapse with para-spinal chloroma 5 years after 1st myeloablative HCT	2	Disease progression. Removed from study before 4th infusion
2	F	51	AML, 2nd HCT for relapse 9 years after 1st myeloablative HCT. 16% blasts at HCT.	2	T-cell persistence. Decrease of MRD-> in CR 2 years after HCT.
3	M	49	AML with complex cytogenetics,received HCT after 2nd CR	1	Poor T-cell persistence. In CR 2 years after HCT
4	M	25	AML, relapse with medullary and extra medullary disease after 2nd HCT	2	Persistent T cells. Chloroma relapse 1 year after infusion.
5	M	49	AML, disease progression after HCT (70% blasts in PB at time of T cell infusion)	2	Disease progression. Off study at 6 days, and expired 12 days after 1st infusion.
6	F	20	AML, second HCT for relapse 2 years after 1st myeloablative HCT	2	Poor T-cell persistence. CR 15 months after HCT.
7	F	33	AML, MRD after myeloablative HCT	2	Progressive disease, deceased.
8	F	63	AML, received HCT in CR2. MRD early after transplant	2	Persistent T-cells, Ongoing MRD (pursued additional therapy - azacitidine).
9	F	67	AML, received HCT in CR2. Relapse early after transplant	2	Poor T-cell persistence, progressive disease.
10	F	64	high-risk AML, received HCT in CR1.	1	Persistent T-cells,CR 6 months after HCT.
11	F	59	High risk AML, received HCT in CR1.	1	Persistent T-cells, CR 1 year after HCT.
12	F	55	HCT in CR1, high risk AML.	1	Persistent T-cells, CR 9 months after transplant
13	F	59	high-risk AML, HCT in CR.	2	Persistent T-cells, CR 7 months after HCT
14	M	17	AML, 2nd HCT for relapse 5 years after first HCT.	1	Poor T-cell persistence, MRD -> received Azacitidine
15	F	69	MDS -> AML, second HCT for relapse 1 year after 1st HCT	2	Poor T-cell persistence, Progressive disease.
16	M	65	MDS-> AML, 5.5% blasts at HCT.	1	Poor T-cell persistence, CR 5 months after HCT.

Overall, 21 of 34 T-cell infusions represented the maximum target dose of 1×10^{10} WT1-specific CTL/m^2, and of those, 8 infusions were followed by low-dose s.c. IL-2. Seven patients (Pts 3, 6, 10, 11, 12, 13, 16) who were at very high risk of relapse after the transplant received the WT1- T-cell infusions while in CR and remain disease free 24, 15, 6, 6, 9, 7 and 5 months after HCT respectively. One of these patients (Pt 4), relapsed 1 year after T-cell transfer with an extramedullary chloroma. Four patients (Pts 2, 8, 9 and 14) had MRD at the time they received T-cells: Pt 2 cleared MRD after receiving the fourth infusion, Pts 8 and 14 continue to have low-level MRD 8 and 5 months respectively after receiving their latest infusion and Pt 9 progressed. Three patients (Pts. 1, 5 and 7) were taken off study due to progressive disease before receiving the 4^{th}, 2^{nd} and 1^{st} infusions respectively; one due to refractory progressing chloroma, one due to rapidly increasing peripheral blasts constituting 70% of total white blood cells at the time of the first infusion and one due to the start of an alternate treatment (azacitidine). Patient 1 went on to receive a second transplant, Patient 5 was placed on comfort care and died 6 days later, and Pt 7 died from progressive disease several weeks after starting azacitidine.

Patients who received cells generated from EBV-specific cells generally demonstrated a high-level of persistence: Of the first 14 treated patients analyzed, 8 patients who could be followed beyond 4 weeks after their latest infusion and who received EBV transduced cells, 6 had persistent frequencies >3% (range 3-60% of CD8 T-cells) for an average follow-up of 27 weeks (range 12-52 weeks) (**Figure 5A**) which represented ~30-200 cells/mcl. (**Figure 5B**). Patient 4 received the lowest dose of 1×10^9, and his cells have remained detectable in the blood at frequencies of 6% to 8% of total CD8 T cells. Due of safety concerns, patients enrolled on either arm of the study cannot receive cells if their pre-existing WT1-specific $CD8^+$ T-cell frequency is no less than 3% of total CD8 T cells. Therefore, at this time, Patients 4, 8, 10, 11, and 12 have not received a second infusion.

Of the 3 pts (Pts 2, 6 and 8) who received CMV-transduced cells, Pt 2 demonstrated persistence of theTCR_{C4}-transduced cells for 39 weeks (ongoing) at frequencies well below 1%. Pts 6 and 8 had no persistence beyond 4 and 2 weeks after their latest infusion respectively.



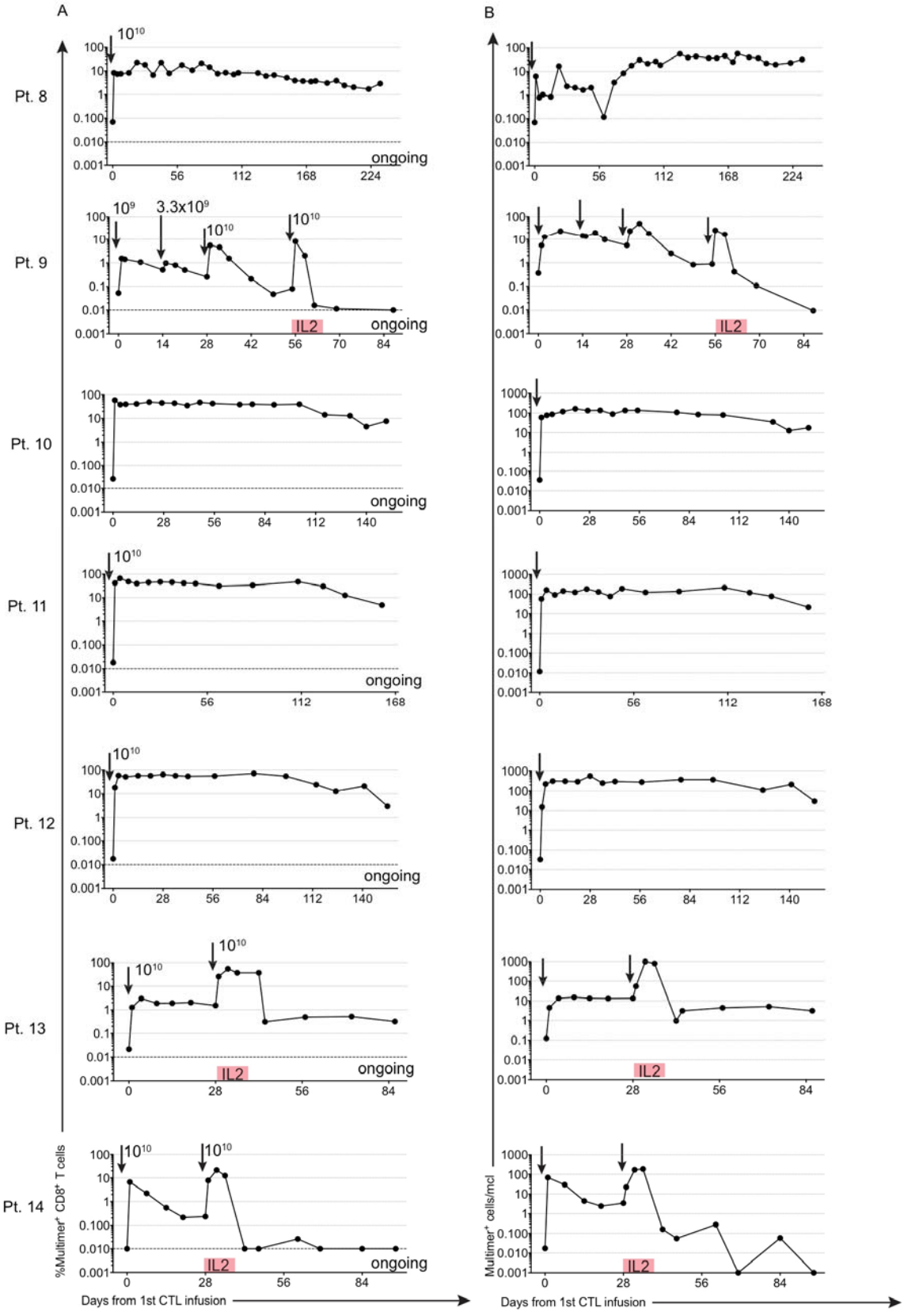


Figure 5A and B. Mean frequencies of infused T cells

3.H.3 Toxicities observed on protocol #2498

All AEs were evaluated starting from the time of the first infusion to 30 days after the patients had taken the last dose of s.c. IL-2 for the first 7 patients then only AEs that were above or equal to a Grade 3 were recorded for subsequent patients. AEs that were deemed possibly, probably, or likely related were collected and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 (NCI CTCAE v4.0) (**Table 2**).

Table 2. Adverse events

Categories	NCI CTCAE v4.0*	Grade 1	Grade 2	Grade 3	Grade 4
Cytokine Release Syndrome	Fever	4	3	2	
	Chills	2	1	1	
	Generalized aches/pain/headache	10	2		
	Fatigue	1	1		
	Tachypnea	4			
	Hypotension	5	2	2	
	Sinus tachycardia	3	2		
	Sinus bradycardia	1			
	Nausea	3	3		
	Vomiting	1			
	Diarrhea	1			
Hematological Abnormalities	Lymphopenia	3	5	8	1
	Anemia	1		3	
	Thrombocytopenia	1	2	3	
	Lymphocyte count increased		2		
Chemistry Abnormalities	ALT increased	6	2		
	AST increased	8			
	Alkaline phosphatase increased	4			
	Creatinine increased	3			
	Hypoalbuminemia	4			
	Hypocalcemia	7			
	Hypomagnesemia	1			
	Hyponatriema	5			
	Hypokaliemia	3			

Miscellaneous	Dry eyes		2		
	Decreased respiratory rate	4			
	Maculo-Papular Rash			1	

*National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0.

Expected transient symptoms were observed due to a **cytokine release syndrome** (CRS) associated with activation of large numbers of antigen-specific CTL transferred into patients with targets expressing the antigen or low-dose s.c. IL-2, or both. Specifically, all patients experienced fevers (≥ 38.3°C) with or without chills with one infusion at most. Blood cultures were all negative for bacterial or fungal growth. Many patients also experienced no greater than grade 2 generalized aches, tachycardia or bradycardia, and digestive tract symptoms. With immediate management by administration of the antihistamine diphenhydramine and acetaminophen, plus the narcotic meperidine for chills, all symptoms resolved within 24 hours. The most severe CRS was observed in one patient who experienced transient grade 3 hypotension during the T-cell infusion that rapidly responded to i.v. fluids. A grade 1 decrease in respiratory rate was observed in 2 patients during the 24 hours after the T-cell infusions, but was associated with administration of meperidine for CRS. All side effects were managed on the general hospital ward without ICU support.

The **hematological abnormality** most commonly encountered was lymphopenia, which is a predictable, transient side effect of T-cell infusions presumably reflecting redistribution of peripheral lymphocytes [10,99]. The temporary drop in total lymphocyte counts returned to pre-infusion levels within 7 to 11 days in all patients. One case of grade 1 anemia and 2 cases of lymphocytosis were observed during the time the patient was receiving T-cell infusions, but did not occur immediately after infusions and the relationship to the T-cell infusions remains unclear. Thrombocytopenia is very common in the first year after allogeneic transplant [100], and all patients treated to date on this study started the T-cell infusion with thrombocytopenia. Overall, we observed a general upward trend in platelet counts during infusions (average platelet counts before infusion: 78 000/µl, after infusions: 140 000/µl), but 3 of 6 evaluable patients had transient drops in platelet counts immediately after infusions most of which did not reach levels associated with a toxicity grade (**Figure 6**).

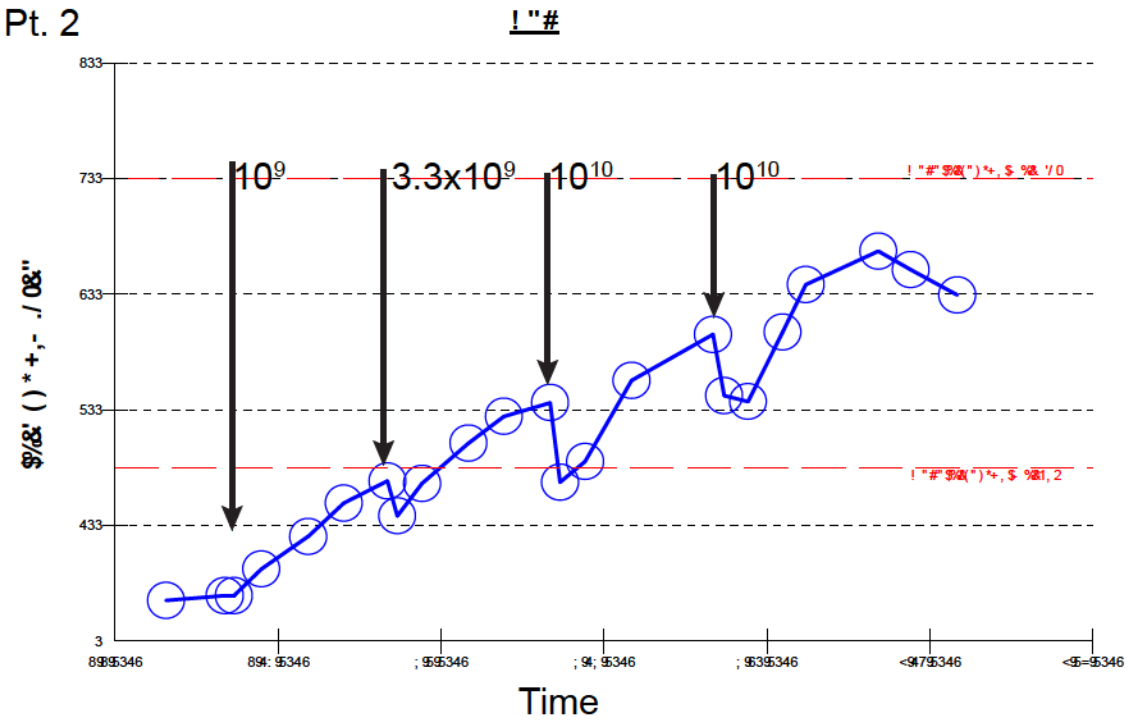


Figure 6. Kinetics of platelet counts after infusions (Pt. 2)

Patient 4 received one T-cell infusion at a dose of 1 × 10^9 cells/m^2 and his platelet count gradually decreased from 95 000 (grade 1) to 45 000 platelets/µl (grade 3) 8 weeks after the infusion, then stabilized at 50 000 to 55 000 at 11 weeks after the infusion, with persisting $WT1^+$ $CD8^+$ T cells still representing approximately 5% to 7% of peripheral CD8 T cells. Marrow evaluation performed at 10 weeks revealed normal megakaryocyte maturation without evidence of infiltrating transferred T cells. Although a relationship to the infusions cannot be formally excluded, the more likely etiology of the decreased platelet count in this patient was the concurrent use of the antifungal Posaconazole, which is known to cause thrombocytopenia in up to 30% of patients. Grade 1 and 2 transient **electrolyte and liver function abnormalities** were also observed. One patient developed transient grade 2 transaminitis directly after the third T-cell infusion, and this resolved without treatment within 7 days. The relationship of other observed metabolic abnormalities to the T-cell infusions could not be excluded, but were clinically perceived to more likely reflect concurrent and common post-HCT etiologies, including side effects resulting from medications (eg, electrolyte abnormalities are common side effects of calcineurin inhibitors and antifungals, and elevated liver enzymes are a common side effect of antifungals), poor nutritional status

(hypoalbuminemia), and secondary infections. One patient developed symptoms of classic chronic graft-versus-host disease (GVHD) involving the eyes (dryness) during the observation period, but no symptoms of acute GVHD during or after T-cell infusions were observed.

3.H.4 Protocol #2498: Conclusions to date

Because toxicities are best evaluated in patients with persistent transferred cells, to date, WT1-specific T cells were detectable in 14 patients for a cumulative total of 224 weeks with frequencies reaching 60% of total CD8 and 998 cells/μl. No durable toxicities to tissues expressing physiological levels of WT1 (including cells of the hematopoietic, urogenital and renal systems, pleura, or pericardium) were detected during the monitoring period. Therefore, adoptive transfer of doses up to 1×10^{10} cells/m^2 of donor-derived virus-specific cells transduced with the WT1-specific TCR_{C4} did not appear to injure normal tissues expressing low physiologic levels of WT1, did not cause acute GVHD, and CRS symptoms induced by the infusion of high numbers of antigen-specific cells were readily managed in this preliminary series.

3.I. *Rational for the proposed cell dose*

Safety of adoptive transfer of doses up to 1×10^{10} cells/m^2 of donor-derived virus-specific cells transduced with the WT1-specific TCR_{C4} has been demonstrated. In the proposed trial, patients will receive a first dose of 1×10^{9} cells/m^2, which is 1 log less than the dose currently administered in the leukemia trial (**Protocol #2498**). They will then receive a second dose of 1×10^{10} cells/m^2, preceded by CY 300mg/m^2 for 2 consecutive days, and followed by low-dose s.c. IL-2 (Arm 1, Stage 1). Although the possibility of inducing tissue toxicity in the proposed trial cannot be excluded, there is no evidence for such toxicity in our current ongoing trial with the same TCR or in our previous trial with WT1-specific CD8 T-cell clones.

3.J. Rationale for the Use of Cyclophosphamide Prior to WT1-specific T-Cell Infusions

The effectiveness of immunotherapy may be enhanced when combined with conventional cytotoxic agents, cyclophosphamide (CY) being the most extensively studied drug [101]. Since the original observation almost 40 years ago that CY could augment immune responses [102],

this effect has been evaluated in experimental animals and clinical studies at doses ranging from 40 to more than 6 000 mg/m^2 [103-107]. In human studies, CY potentiated delayed-type hypersensitivity (DTH) responses to a vaccine in patients with metastatic cancer at doses as low as 300 mg/m^2 [108-111]. Studies have suggested that low doses of CY (200-400 mg/m^2) can selectively deplete suppressor activity whereas higher doses (1000-4000 mg/m^2) induced a more global lympho-depletion but may have had a beneficial 'bystander' effect promoting preferential homeostatic expansion of responding T cells in lymphodepleted hosts [106,110].

3.K. The Use of IL-2 in Adoptive T-Cell Therapy

In our prior clinical studies, adoptively transferred CTL clones exhibited a median survival of 6.6 +/- 0.8 days in the absence of IL-2. The addition of a 14-day course of low-dose IL-2 (250 000 U/m^2 twice daily) following T-cell infusion yielded a median CTL survival of 16.8 +/- 1.6 days, which was significantly longer than that observed in infusions administered to the same patient without IL-2 [98]. It was apparent, however, that the frequency of transferred CTL *in vivo* began to drop before the end of the 14-day course, suggesting continuing IL-2 administration beyond this time may be nonproductive or even counter-productive as it may concurrently enhance expansion of CD4 T-regulatory cells.

IL-2 is a cytokine secreted by activated T cells, and has a crucial role in the generation of an effective immune response. IL-2 promotes the activation and proliferation of antigen-specific T cells via the high-affinity three-chain (αβγ) IL-2 receptor, with the α-chain induced following TCR triggering. High concentrations of IL-2 can also activate resting natural killer (NK) cells and T cells via the constitutively expressed intermediate affinity two-chain (βγ) IL-2 receptor. Studies in cancer patients have evaluated therapies with IL-2 alone, with adoptively transferred lymphokine-activated killer (LAK) cells generated by culture in high dose IL-2, or with tumor infiltrating lymphocytes (TIL) expanded by culture with IL-2 [112].

These studies used high doses (up to 50 × 10^6 units every 8 hours) of i.v. or s.c. IL-2. Although clinical responses were observed in a fraction of patients with renal cell carcinoma or melanoma, severe toxicity was a significant problem. The IL-2 plasma levels obtained following high-dose IL-2 administration exceed the concentrations needed to saturate intermediate affinity receptors, and can therefore induce non-specific widespread activation

of NK cells and T cells that can likely mediate most or all of the observed toxicities via cytokine release and lysis of normal cells. As $CD8^+$ T cells activated *in vivo* by specific recognition of a target antigen are induced to express high-affinity IL-2 receptors and are therefore responsive to very low concentrations of IL-2, the doses necessary to augment survival and *in vivo* persistence of antigen-specific transferred T cells may be much lower than the doses that induce toxicity.

Studies in cancer patients and HIV seropositive individuals have investigated the immunomodulatory effects of administering lower doses of IL-2. Doses of 1.25×10^5 to 5×10^5 U/m^2/day administered s.c. are well tolerated for up to 84 days[113]. In cancer patients, there is little evidence for anti-tumor activity from administration of low-dose IL-2 alone. However, studies from our group of melanoma patients do suggest that the persistence, *in vivo* function, and anti-tumor activity of adoptively transferred $CD8^+$ melanoma-specific CTL clones can be significantly enhanced by daily s.c. administration of low doses of IL-2 for 14 days following T-cell transfer [114].

The dose of IL-2 that will be used in this study was chosen based on its predicted ability to saturate high-affinity IL-2 receptors and sustain CD8 CTL activity and survival with minimal toxicity [98]. Patients previously treated with **donor-derived $CD8^+$ T-cell clones after HCT**[10] and patients treated on **BB-IND 15130 – FHCRC Protocol 2498,** all received the same dosing of IL-2 planned for this trial. IL-2 has been well tolerated, with no evidence of induction of GVHD from non-specific T-cell activation.

3.L. Rationale for Additional T Cell Infusions

Previous studies in the autochthonous pancreatic solid tumor murine model with infusion of murine cells transduced with a high affinity T cell receptor that recognizes the pancreatic over-expressed antigen mesothelin (Stromnes et al., 2015), have demonstrated that the infused cells become progressively dysfunctional when they reach the tumor micro-environment and loose their anti-tumor effect. To overcome this limitation, serial infusions of the same T cells successfully resulted in a near doubling of the survival of the mice without additional toxicities. These murine studies of a high affinity TCR that recognizes a native tumor antigen of mesothelin provides a very similar model to the TCR_{C4}-transduced T cells

in NSCLC and mesothelioma, which are a native high-affinity TCR recognizing $WT1_{126-134}$, a native antigen over-expressed in select tumors.

This data supports the potential for increased clinical benefit from additional infusions of TCR_{C4}-transduced T cells in selected patients who demonstrate clinical benefit and no serious toxicities from the initial, planned T cell infusion.

4. OBJECTIVES

4.A. Primary Objectives

1. Determine the safety, and potential toxicities associated with treating patients with metastatic NSCLC and mesothelioma with polyclonal autologous central memory and naïve $CD8^+$ T cells that have been transduced to express a WT1-specific TCR (Arm 1 and Arm 2).
2. Determine the feasibility of treating patients with metastatic NSCLC and mesothelioma with polyclonal autologous central memory and naïve $CD8^+$ T cells that have been transduced to express a WT1-specific TCR (Arm 1 and Arm 2).
3. Determine and compare the *in vivo* persistence in blood and tumor of transferred polyclonal autologous central memory and naïve $CD8^+$ T cells that have been transduced to express a WT1-specific TCR (Arm 1 and Arm 2).

4.B. Exploratory Objectives

1. Determine the antitumor efficacy for patients with metastatic NSCLC and mesothelioma (Arm 1), as measured by time to progression (TTP) based on the RECIST 1.1 criteria.
2. Determine the *in vivo* functional capacity of adoptively transferred polyclonal autologous $CD8^+$ T cells that have been transduced to express a WT1-specific TCR, and assess the acquisition of phenotypic characteristics associated with T-cell exhaustion (Arm 1 and Arm 2).
3. Determine the migration to tumor sites of adoptively transferred polyclonal autologous $CD8^+$ T cells that have been transduced to express a WT1-specific TCR (Arm 2).

4. Evaluate the tumor response and T-cell infiltration in tumors of patients with stage IIIA NSCLC treated in the neo-adjuvant setting.

5. STUDY ENDPOINTS

5.A. Primary Endpoints

1. Evidence and nature of toxicity based on the NCI CTC AE v4.0 (see **Section 21.A**).
2. Feasibility of T_N and T_{CM} subsets in patients with metastatic NSCLC and mesothelioma (see **Section 21.B**).
3. Comparison of persistence between T_N and T_{CM} groups in blood and localization and persistence in tumor (see **Section 21.C**).

5.B. Exploratory Endpoints

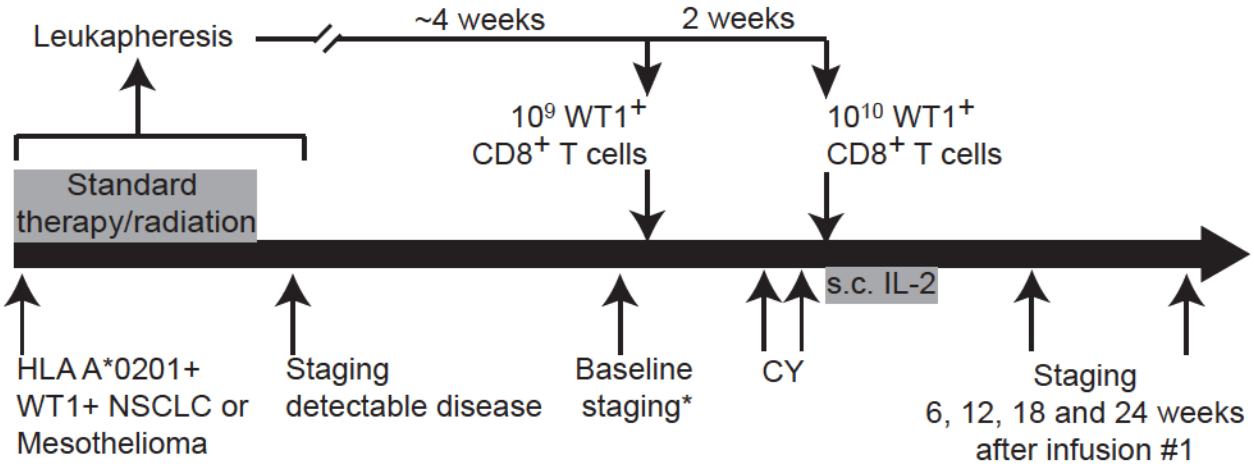
1. Time to progression based on response evaluation criteria in solid tumors (RECIST) 1.1 criteria and RECIST 1.1 mesothelioma modified (see **Section 21.D**).
2. Maintenance of functional capacity and acquisition of phenotypic characteristics associated with T-cell exhaustion of transferred T cells (see **Section 15.F**).
3. Comparison between T_N and T_{CM} groups of the frequency of transferred T cells at biopsied tumor sites.

6. STUDY DESIGN

The proposed study is a Phase I/II trial aimed at treating at least 12 but up to 20 individuals over 3 years with NSCLC or mesothelioma in the advanced metastatic setting. Due to safety concerns, enrollment and treatment is organized in a step-wise manner:

Arm 1 Stage 1 will include at least 6 patients with recurrent or metastatic Stage IV NSCLC (or Stage IIIB whose disease is resistant or who are ineligible for curative-intent therapy) who have measurable disease after standard therapy or radiation or both, or whose disease is resistant to PD-1/PD-L1 blocking agents or advanced mesothelioma who have unresectable disease and have received at least one line of treatment. However, because the incidence mesothelioma is less than NSCLC, it is not known whether these patients can be recruited. Patients will undergo a leukapheresis (or a 120 ml blood draw if a

leukapheresis cannot be performed) to collect peripheral blood mononuclear cells (PBMC) - (Please refer to **Sections 15.B**. **and 15.C** for the timing of the leukapheresis). From the PBMC collected, WT1-specific T cells will be generated and patients will be planned to receive both naïve and central memory autologous polyclonal $CD8^+$ T cells transduced with a characterized high-affinity WT1-specific TCR (TCR_{C4}) with a 1:1 ratio. In cases where cells cannot be generated from the T_N pool or the T_{CM} pool, the infused cells may be generated from the T_N pool or the T_{CM} pool only. Patients who have not received recent therapeutic radiation will receive **a first dose of 1×10^9 WT1-specific cells/m^2 on Day 0 (this test dose is 1 log less than the doses used in protocol #2498 which have resulted safe)** as soon as 4 weeks after the leukapheresis or blood draw (depending on the availability of the T-cell product and the clinical course of the patient). This will be followed by 2 days of low-dose CY 300 mg/m^2 on Days 11 and 12 and by **1×10^{10} WT1-specific cells/m^2 on Day 14**. The higher dose will be followed by low-dose s.c. IL-2 at 250 000 IU/m^2 twice daily for 14 days (**Figure 7**). If the final T cell product does not meet the target cell dose specification, the cell product may still be infused.



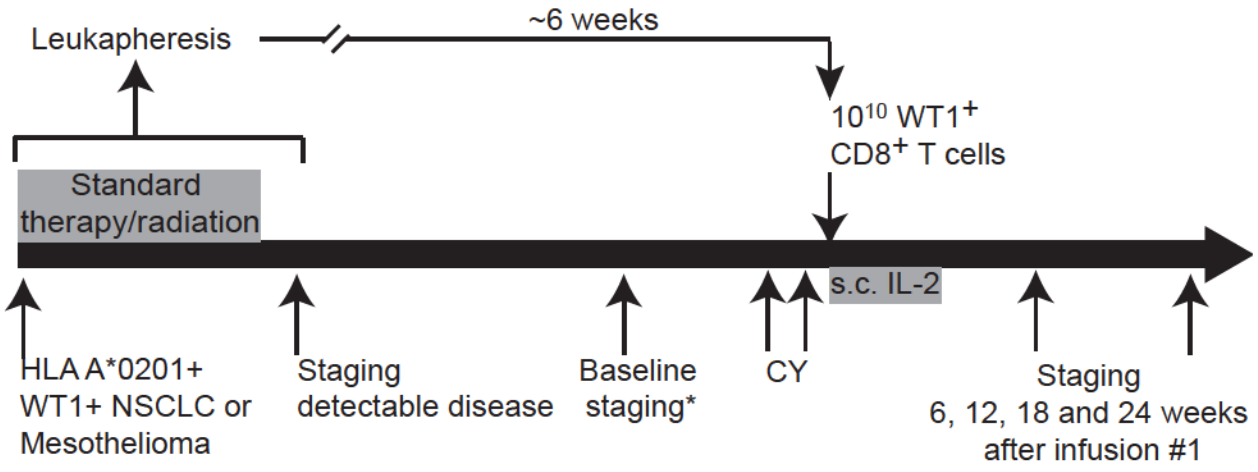
*Only if staging was not performed in the previous 4 weeks.

Figure 7. Plan of Treatment – Arm 1 Stage 1

Staging studies to assess efficacy will be performed at 6, 12, 18 and 24weeks after the first infusion. Patients who have received radiation to the chest/lung tissue will be eligible to receive WT1-specific T cells 90 days after completion of radiation due to the risk of developing radiation-induced pneumonitis at earlier times.

After 6 patients have received polyclonal TCR_{C4}-transduced $CD8^+$ T cells on Arm 1 Stage 1, the DSBM will meet to determine whether the toxicities of the treatment are acceptable and safe so that enrollment on **Arm 1 Stage 2** and **Arm 2** can proceed (see **Section 19.C**).

Arm 1 Stage 2 will include 6, but up to 10, patients with Stage IV NSCLC (or Stage III whose disease is resistant or who are ineligible for curative-intent therapy) who have measurable disease after standard therapy or radiation or both, or whose disease is resistant to PD-1/PD-L1 blocking agents, and patients with advanced mesothelioma who have unresectable disease and have received at least one line of treatment.. Their treatment will consist of 2 days of low-dose CY 300 mg/m^2 on Days -3 and -2 and the infusion of **1×10^{10} WT1-specific cells/m^2** on Day 0, followed by low-dose s.c. IL-2 at 250 000 IU/m^2 twice daily for 14 days (**Figure 8**). The cell product may be infused even if the target cell dose specification is not reached.



*Only if staging was not performed in the previous 4 weeks.

Figure 8. Plan of Treatment – Arm 1 Stage 2

Arm 2 will include up to 4 patients who have either potentially resectable Stage III NSCLC or surgically operable mesothelioma and are candidates for neo-adjuvant chemotherapy prior to resection surgery. Identified patients will be screened for the HLA A*0201 genotype and whether tissue is available for expression of WT1 by their tumor. Patients will undergo a leukapheresis (or a 120 ml blood draw if a leukapheresis cannot be performed) before

starting neo-adjuvant chemotherapy from which the WT1-specific T cells will be generated. These patients will receive 1 × 10^{10} WT1-specific naïve T cells/m^2 more than 24 and less than 96 hours after the last dose of chemotherapy to allow for drug clearance (**Figure 9**). If the final T cell product cannot be formulated to meet the target cell dose specification, the cell product may still be infused. Surgery will not be delayed due to the infusion and will occur within 3 to 4 weeks after the infusion. The resection specimen will be analyzed for the presence of infiltrating WT1-specific T cells, as well as other immunologic parameters (eg, presence of regulatory T cells, expression of PD-L1 by tumor, etc.). All patients will be monitored on study for at least 6 months after the infusion with re-staging studies performed 6, 12, 18 and 24 weeks after the first infusion, and then as clinically indicated.

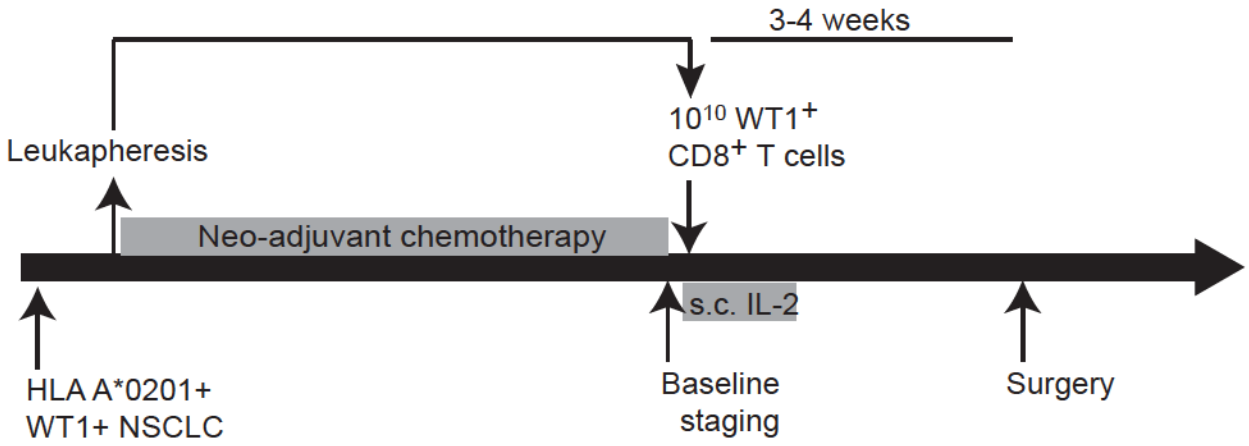


Figure 9. Plan of Treatment – Arm 2

7. PATIENT SELECTION

7.A. Eligibility for Enrollment/Screening (Arms 1 and 2)

1. Histopathological documentation of NSCLC or mesothelioma.
2. 18 years of age or older.
3. Patients must be able to give informed consent.
4. Patients must be able to provide blood and tumor samples and undergo the procedures required for this protocol.
5. **Arm 2 ONLY:** Surgically operable NSCLC or mesothelioma.

7.B. Exclusion for Enrollment/Screening (Arms 1 and 2)

1. Eastern Cooperative Oncology Group (ECOG) performance status ≥ 2 (see **Appendix A)**.
2. Active autoimmune disease (eg, systemic lupus erythematosus, vasculitis, infiltrating lung disease, inflammatory bowel disease) in which possible progression during treatment would be considered unacceptable by the investigators.
3. Any condition or organ toxicity deemed by the Principal Investigator (PI) or the attending physician to place the patient at unacceptable risk for treatment on the protocol.
4. Men or women of reproductive ability who are unwilling to use effective contraception or abstinence. Women of childbearing potential must have a negative urine pregnancy test within 2 weeks prior to first infusion.
5. Pregnant women and nursing mothers will be eligible for **screening only** to test HLA type by saliva or buccal swab and WT1 expression from previously collected tissue sample.
6. Clinically significant and ongoing immune suppression including, but not limited to, systemic immunosuppressive agents such as cyclosporine or corticosteroids, chronic lymphocytic leukemia (CLL), uncontrolled human immunodeficiency virus (HIV) infection, or solid organ transplantation.

7.C. Eligibility for Treatment on Arm 1

1. Patients must express HLA-A*0201.
2. Evidence of WT1 tumor expression.
3. Patients must have received at least one line of therapy for NSCLC or mesothelioma or previously documented to have declined therapy.
4. NSCLC patients with a mutation in EGFR or ALK must have demonstrated progression or intolerance to at least one of the corresponding targeted therapies (for example erlotinib or crizotinib).
5. Bi-dimensionally measurable disease by palpation, clinical exam, or radiographic

imaging (X-ray, CT scan, PET scan, MRI, or ultrasound).

6. Ninety days must have passed since the last doses of radiation or chemoradiation treatment involving lung tissue or thorax prior to T-cell infusion (to avoid confounding pneumonitis [115]).
7. Patients treated with prior immunotherapy including and not limited to vaccines, cytokines, T-cell stimulating agents, CTLA4 inhibitors and PD-1 check point inhibitors are allowed on therapy provided they did not have any severe grade 4 toxicities due to prior therapy and any toxicities due to prior therapy should have resolved, if resolvable to less than or equal to grade 1.

7.D. Eligibility for Treatment on Arm 2

1. Patients must express HLA-A*0201.
2. Evidence of WT1 tumor expression
3. Ninety days must have passed since the last definitive doses of radiation or chemoradiation treatment prior to T-cell infusion (to avoid confounding pneumonitis)[115].

7.E. Exclusion for Treatment (Arms 1 and 2)

1. Exclusions for the Leukapheresis procedure (this can be performed at a later time of symptoms resolve):
    a. Infection, with or without antibiotic treatment.
    b. Recent hepatitis exposure (hepatitis B or C antigenemia)
    c. Pregnancy or nursing.
    d. HIV or HTLV infection.
    e. Positive result on Standard Test for Syphilis (STS)
2. Unable to generate antigen-specific WT1-specific $CD8^+$ T cells for infusions. However, the patient will have the option to receive WT1-specific T-cells if a lower than planned number of cells is available.

3. Documented infections or known oral temperature > 38.2 °C fewer than 72 hours prior to receiving study treatment or systemic infection requiring chronic maintenance. The start of treatment may be delayed.
4. Systemic steroids should be stopped 2 weeks before the start of treatment. Topical and inhaled steroids are allowed.
5. Untreated CNS metastasis that are > 1cm or symptomatic are not allowed. (Patients with CNS metastases >1cm or symptomatic that have been treated and demonstrated to be radiologically and clinically stable for at least 4 weeks are allowed.)
6. Complete blood count (CBC) profile prior to treatment:

   White blood cells (WBC) < 2,000/µl
   Hemoglobin (Hb) < 8 g/dL
   Absolute neutrophil count (ANC) < 1,000/µl
   Platelets < 50,000/µl
7. New York Heart Association functional class III-IV heart failure, symptomatic pericardial effusion, stable or unstable angina, symptoms of coronary artery disease (CAD), congestive heart failure, clinically significant hypotension or history of an ejection fraction of ≤ 30% (echocardiogram or Multi-Gated Acquisition Scan [MUGA]).
8. Clinically significant pulmonary dysfunction, as determined by medical history and physical exam. Patients so identified will undergo pulmonary functions testing and those with forced expiratory volume in 1 second (FEV1) < 2.0 L or diffusion capacity for carbon monoxide (DL_{CO}) (corrected for Hb) < 50% will be excluded.
9. Creatinine >1.5 × the upper limit of normal.
10. Significant liver dysfunction: aspartate aminotransferase / alanine aminotransferase (AST/ALT) > 5 × upper limits of normal (ULN) and or Bilirubin > 3 × ULN that cannot be attributed to NSCLC metastasis.
11. HIV or HTLV infection.

8. CONSENTING

A **screening conference** will be held with the patient. The PI or a delegated representative will discuss this study and alternative treatments available for metastatic NSCLC. All known risks and potential hazards of treatment with WT1-specific transduced memory and naïve polyclonal $CD8^+$ T cells and low-dose s.c. IL-2 will be discussed. Informed consent will be obtained from the patient using forms approved by the Institutional Review Board (IRB) of the FHCRC. After the **screening consent form** is signed, the patient may then be HLA typed and the patient's tumor may be evaluated for WT1 expression.

When **tumor HLA type and WT1 expression has been confirmed**, a second conference will be held with the patient to confirm participation in the treatment study. In this case, the **treatment consent form** will be signed. The patient may then undergo a leukapheresis to collect the peripheral blood mononuclear cells (PBMC) that can be used to generate the cellular product (Please see **Sections 14.B and C** for the timing of the leukapheresis procedure. The patient will also be informed that it is possible to withdraw from the study at any time and for any reason without jeopardizing future treatment. In accordance with federal regulations (21 CFR 312), the patient must sign the IRB-approved treatment consent form. Patients may sign the screening consent form at any time after their diagnosis of NSCLC or mesothelioma.

9. PROTOCOL REGISTRATION

Patients will be assigned to the protocol by the Trial Coordinator who will register the patient with the Registration Office, (206) 667-4728, between 8:30 am and 4:00 pm, Monday through Friday. After hours, the Registration Office can be reached by paging (206) 995-7437.

10. PROCEDURE TO OBTAIN PBMC FOR GENERATION OF WT1-SPECIFIC $CD8^+$ T CELLS

The preferred source for $CD8^+$ T-cell isolation is a non-granulocyte colony-stimulating factor (non-GCSF) -mobilized 6- or 12-liter leukapheresis planned after the patient has signed the informed consent form and the tumor has tested positive for WT1 expression. The leukapheresis can be performed at any time after the diagnosis of WT1-expressing NSCLC or WT1 expressing Mesothelioma.

A. Patients will not be considered for leukapheresis if they have a medical condition precluding leukapheresis, but may undergo leukapheresis at a later time if condition resolves.

   *Exclusions include (see also **Section 7.E**)*:

   - Infection, with or without antibiotic treatment.
   - Recent hepatitis exposure, hepatitis B antigenemia, or hepatitis C antibody positivity.
   - Pregnancy or nursing.
   - HIV or HTLV infection.
   - Positive result on Standard Test for Syphilis (STS)

B. At the discretion of the PI an additional 6 or 12 liter leukapheresis may be repeated if insufficient cells are obtained.
C. If the patient is unable or unwilling to undergo separate leukapheresis, 120 mL of peripheral blood can alternatively be drawn for generation of the T cells.

PBMC may be cryopreserved and used to generate T cells for this study.

11. GENERATION OF WT1-SPECIFIC $CD8^+$ T CELLS

All products administered will be derived from the peripheral blood lymphocytes of a patient with an established diagnosis of NSCLC or Mesothelioma, contain autologous transduced WT1-specific $CD8^+$ T cells derived from the T_{CM} and T_N subsets, and will be generated using clinical-grade reagents according to Standard Operating Procedures (SOPs) in the Good Manufacturing Practice (GMP)-grade Cell Processing Facility (CPF) of the FHCRC. Methods employed to generate and qualify products for infusion are outlined in **BB-IND 15130** (Sponsor Dr. Aude Chapuis). T cells demonstrating antigen-specificity are further qualified using mycoplasma, fungal, and bacterial sterility testing. In cases where cells cannot be generated from the T_N pool or the T_{CM} pool, the infused cells may be generated from the T_N pool or the T_{CM} pool only.

12. HANDLING OF T CELL PRODUCTS BEFORE INFUSION

For each infusion dose, the gene-modified cell product is formulated at the desired cell dose in a final volume of 250 ml. The final product will be prepared and labeled according to SOPs in the CPF. After release from the CPF, the cell product will be transported to the infusion facility by a protocol-delegated staff member. During the time of transportation the cell product will be kept in a cooler with a cool pack. A nurse will then administer the cells to the patient approximately over 1 to 2 hours or longer if clinically indicated (as described in **Section 14.E**).

13. OTHER STUDY AGENTS

13.A. Cyclosphosphamide

CY at 300 mg/m^2 will be administered intravenously for 2 consecutive days. Standard Practice Policy guidelines will be followed and its administration will be completed at least 48 hours prior to the T-cell infusion.

13.B. Interleukin-2

IL-2 will be initiated within 6 hours of the T-cell infusion of 10^{10}/m^2. This low-dose IL-2 regimen will be administered at 250 000 U/m^2 s.c. twice daily for 14 days. The patient or the caregiver will be instructed on s.c. self-administration.

14. PLAN OF TREATMENT

A. Initial Evaluation/Screening Consent Visit

Patients will be screened for their eligibility on the study, and the patient will be requested to understand and sign the screening consent during this visit. After signing the screening informed consent form, their blood, saliva or buccal swab will be evaluated for the expression of HLA A:0201 and their tumor will be evaluated for WT1 expression. Screening consent may be conducted by phone at the discretion of the primary investigator.

B. Treatment Consent Visit.

Patients who are HLA A*0201$^+$ and whose tumors express WT1 and who are willing to participate in the treatment protocol will be scheduled for a treatment consent visit. After

signing the treatment consent (specific for either Arm 1 or 2 depending on their clinical condition and planned treatment), a leukapheresis may be scheduled before, during, or after the patients' current treatment course at the discretion of the PI and the attending physician.

D. Leukapheresis Visit

Patients consenting to this study will undergo leukapheresis at a time prior to anticipated interval systemic therapy or, if the patient is receiving treatment, at least 2 weeks from the last treatment cycle.

D. Cyclophosphamide Infusion Visits

This visit will be scheduled at least 4 weeks after the leukapheresis visit to enable the generation of a T-cell product and when the patient meets treatment criteria on Arms 1 or 2. The CY will be administered intravenously 3 and 2 days prior to T-cell infusion as an outpatient procedure. For women of child-bearing potential, the confirmation of the absence of pregnancy will be required within 2 weeks prior to infusion (Arm 1 Stage 2).

E. T-Cell Infusion

T-cell infusions will be given at the SCCA Immunotherapy Clinic or as an inpatient at the UWMC. For women of child-bearing potential, the confirmation of the absence of pregnancy will be required within two weeks prior to the first infusion. All patients will be admitted for an overnight stay at the GCRC or UWMC. T cells will be infused intravenously at a rate of no greater than 250 cc/hour through an 18- or 20-gauge catheter inserted into a peripheral vein or through a central catheter. The infusion bag will be gently mixed periodically during the infusion. Subjects will have vital signs obtained at the start of the infusion, every 15 minutes during the infusion, and then as described in **Section 15.D**, Subjects will have oxygen saturation measured by pulse oximetry pre-infusion, immediately post-infusion, and 2 hours post infusion. The patients will be released the next morning if no AEs requiring more intensive monitoring or continued hospitalization have occurred (see **Section 16**).

F. Low-dose S.C. IL-2

After the cell infusion of $10^{10}/m^2$, patients will receive twice daily s.c. injections of recombinant IL-2 at a dose of **2.5×10^5 U/m^2 every 12 hours for 14 days** (28 doses) with the first dose starting within 6 hours after completing the T-cell infusion. Because all patients will be hospitalized overnight following the T-cell infusion, at least 2 doses of IL-2 will be administered in the hospital. Patients who demonstrate no immediate significant toxicities from the T-cell infusion and are discharged from the hospital will be trained (if possible) to self-administer the subsequent IL-2 doses as an outpatient. IL-2 therapy will be discontinued in any patient developing grade 3 or greater treatment-related toxicities (see **Section 16**) while receiving daily s.c. IL-2 injections.

15. EVALUATION

See **Protocol Appendix B** for a summary of patient evaluation before, during, and after T-cell therapy. The dates listed on the study calendar are approximate as it is anticipated that many patients will reside out of the area and may not always be able to follow the precise time points as dictated by the protocol. Potential patients will initially be identified and recruited through the Seattle Cancer Care Alliance (SCCA) Lung Cancer Clinic. Informed consent forms will be discussed and signed in the presence of a Good Clinical Practices (GCP)-trained investigator. Patients who are found eligible to participate in this protocol will be followed closely by a multidisciplinary oncology team that includes medical oncologists and radiation oncologists (as appropriate). The infusion of WT1-specific T cells will be performed within 4 weeks of the last NSCLC re-staging studies.

15.A. Patient Evaluation during the Screening Visit

Patients will have a saliva collection, buccal swab or blood draw performed. Eligibility for enrollment and screening will be checked.

15.B. Patient Evaluation during the Pre-Treatment Visit

Patients will have a history taken, ECOG performance status determined (**Appendix A**), a physical exam with vital signs performed, a renal and hepatic panel with magnesium and LD and complete blood count as well as blood for research obtained. Eligibility for treatment will be assessed and documented.

15.C. Patient Evaluation at Each Planned Visit

Patients will have a history taken, ECOG performance status determined (**Appendix A**), a physical exam with vital signs performed, a a renal and hepatic panel with magnesium and LD and complete blood count obtained at planned visits including prior to the T-cell infusion, 1, 3 and 7 days after each infusion, and weekly up to 4 weeks after each infusion (**Appendix B**).

15.D. Patient Evaluation During T-cell Infusions

Blood pressure, heart rate, temperature, respiratory rate, and O_2 saturation (by pulse oximetry) will be recorded at time 0, every 15 minutes during the 1- to 2-hour T-cell infusion, hourly for 2 hours, and then every 4 hours while awake following the T-cell infusion. AEs will be managed by standard medical practice (see also **Section 16**).

15.E. Clinical and Laboratory Evaluation for Toxicity (Primary Endpoint)

The period of monitoring for treatment-related toxicity will start with the first intervention on this study, either T-cell infusions (Arm 1, Stage 1) or CY infusions (Arm 1, Stage 2 and Arm 2), and end after 6 months on the trial, if the patient has not developed or has resolved potential toxicities (see **Section 16 and Appendix B**). The evaluation days listed in Appendix B should be respected as closely as possible. However, because some draws cannot be performed on weekends and it is anticipated that some patients reside outside the Seattle area, we suggest the following guidelines: Daily visits can occur +/- 1 day, weekly visits can occur +/- 3 days and monthly visits can occur +/- 5 days before/after the planned date. In addition, frequency of monitoring may potentially be increased, if indicated, based on the patient's clinical condition.

15.E.1 General Toxicity Assessment

Grade ≥ 3 AEs will be recorded and graded according to the NCI CTCAE v4.0 up to 4 weeks after the last infusion (see **Section 16**). Beyond the 4 week monitoring period after infusion, unexpected illnesses or new malignancies may be identified by the patient's primary oncologist. The study team may provide outside records to the PI to determine whether events are serious, unexpected, and/or related. According to the current FDA

guidance, patients are recommended to undergo at least annual history and physical examination with their primary physician for a minimum of 15 years (**15.K.2**).

To evaluate for potential toxicities, patients will undergo a history and physical exam prior to each infusion, day +1, +7 and 2, 3, 4, 6, 12, 18 and 24 weeks. Laboratory evaluations (renal and hepatic panel with MG and LD and complete blood count) immediately prior to infusions, days +1, +3, +7, and 2, 3, 4, 6, 12, 18 and 24 weeks after each infusion (see **Appendix B**).

A history and physical exam and laboratory evaluations (renal and hepatic panel with MG and LD and complete blood count) at each restaging visit (week 6, 12, 18 and 24).

In order to allow enrollment on Arm 1 Stage 2 and Arm 2 and if no unexpected, serious, and related AEs occur that would warrant an early DSMB meeting, the DSMB will meet at least 4 weeks after the sixth patient has received the last T-cell infusion (see **Section 19**).

15.E.2. Toxicity requiring treatment discontinuation

The occurrence of treatment-related toxicity requiring treatment discontinuation will result in no further infusions being given and also in the administration of glucocorticoids to ablate the transferred T cells (see **Section 16** for details).

15.F. Evaluation of Persistence and Function of Adoptively Transferred T Cells (Primary Endpoint)

15.F.1 Blood Samples

Sixty (60) ml of blood with acid citrate dextrose (yellow top) will be drawn prior to infusion and at selected time points following infusion per section 15.E.1 (see **Appendix B**, blood draw for lab). Samples will be used to evaluate the duration of *in vivo* persistence of T_N and T_{CM} subsets and their function and phenotype. All blood samples will be kept at room temperature and sent to room D3-235, FHCRC. For Arm 1 (Stage 1 and 2 combined), the persistence observed in the T_N group will be compared to the T_{CM} group (see **Section 21.C**).

15.F.2 Tumor samples

Only patients with histologically confirmed $WT1^+$ NSCLC or mesothelioma will be enrolled in the study. If tumor tissue is available from the original diagnostic biopsy or if the patient undergoes an additional biopsy before the start of treatment, we will obtain a specimen for analysis. If the material obtained is paraffin fixed, it may be used to examine the phenotype of the tumor cells (for example, if they express PD-L1 and HLA class I) and the presence and the nature of an endogenous T-cell infiltrate (for example, if cells express CD4, CD8 and PD1).Presence and frequency of T_N and T_{CM} subsets will be assessed by HTTCS. Localization of transferred WT1-specific CTL to tumor sites after treatment will be evaluated in selected patients with surgically accessible disease (disease that is accessible by needle or core biopsy, a malignant aspirate [Arm 1 and 2], or tumor that is surgically excised [Arm 2]). If enough material can be obtained, single cell suspensions will be prepared and stained to identify the transferred CTL by binding the WT1-specific tetramer and expressing the TCR_{C4}-specific Vβ chain (Vβ 17), as well as analyzed for phenotype and function. The frequency of transferred T_N and/or T_{CM} WT1-specific cells will be compared with the peripheral blood by HTTCS to assess whether the cells preferentially accumulate to the tumor site.

15.G. Feasibility Assessment (Primary Endpoint)

All screened patients will be assessed for expression of HLA A*0201 and tumor-expression of WT1, likelihood of generating a T-cell product from PBMC and time required to generate the products (See **Section 21.B**). The feasibility of this approach will be evaluated as:

1. The number of subjects screened who are eligible to receive T-cells.
2. Patients who signed the treatment infused consent and underwent a leukapheresis from which we were not able to generate a product.
3. Time required to generate a TCR_{C4}-transduced T-cell product product.

15.H. Option for Additional T Cell Infusions

We will offer patients the option to undergo additional TCR infusions, if they meet the following eligibility criteria:

1) they have shown evidence of clinical benefit after the initial, planned infusions on this study, demonstrated by either a measureable reduction in tumor burden or a clinical improvement in symptoms, and
2) they have not experienced serious, regimen-related, unexpected toxicities from prior treatment on this study, and
3) there are remaining cells from their leukapheresis to generate an additional infusion product.

Cyclophosphamide administration before the additional cell infusions will not be administered to avoid elimination of previously infused cells, which may still be functional.

Guidelines for the additional T cell infusions:

- Patients will receive a goal of up to 10^{10} TCR_{C4} cells/m^2 every 4 weeks with the same goal ratio of T_N and T_{CM} as the first 2 infusions. If there are insufficient cells to achieve goal dose or the goal ratio, the generated cells may still be infused.
- If no unexpected, regimen-related Grade 3/4 toxicities develop, and the patient continues to experience clinical benefit (as defined by a measurable reduction in tumor burden or improvement in symptoms), this schedule can be maintained until a CR is obtained.

Guidelines for IL-2 administration after the additional T-cell infusion:

- Low-dose IL-2 is planned to be administered with follow-up T cell infusions at the same dosing as the previous T cell infusion (250,000 units/m^2 s.c. twice daily for 14 days).
- If patients develop toxicities specifically associated with IL-2 cycles, or if patients refuse IL-2 (e.g. due to anxiety over ongoing subcutaneous injections), IL-2 administration for the additional T-cell infusions may be discontinued and the T cell infusions continued without IL-2 administration.

15.I. Efficacy Assessment: Assessment of Clinical Responses by RECIST 1.1 Criteria and RECIST 1.1 mesothelioma modified (Exploratory Endpoint)

All patients treated on this protocol will have developed metastatic disease. To assess the potential systemic effects of adoptively transferred T cells, radiographic imaging and clinical assessment of residual disease will be compared with pre-infusion assessment using primarily the RECIST 1.1 criteria [116] and the RECIST 1.1 mesothelioma modified, which takes into consideration the particularities of mesothelioma. Assessment of tumor burden by both RECIST 1.1 and RECIST 1.1 mesothelioma modified (see **Appendix C**) will be performed at 6, 12, 18 and 24 weeks after the first T-cell infusion and then at least once every 2-3 months or as clinically indicated after 24 weeks following the last infusion or until new metastases occur (see **Appendix B**). Assessment of best overall responses and duration of responses will be following the RECIST 1.1 criteria.

15.J. Assessment of the Functional Capacity of Transferred Cells (Exploratory Endpoint)

To evaluate the direct *ex vivo* function of the transferred cells, tetramer$^+$ cells from collected PBMC, if detectable and available in sufficient number, will be evaluated for production of intracellular cytokines including interferon-gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and IL-2, in response to cognate antigen using flow cytometric intracellular cytokine assays combined with surface staining.

Intranuclear Ki-67 expression will be assessed on recovered tetramer$^+$ T cells as a surrogate marker of *in vivo* proliferation of the transferred cells. Finally, based on available cell numbers, the *ex vivo* proliferative capacity of cells after infusion will be assessed by labeling cells with carboxyfluorescein succinimidyl ester (CFSE) dye and measuring dilution in response to peptide stimulation.

The phenotype of tetramer$^+$ antigen-specific cells in the peripheral blood will be assessed using established immunophenotyping panels which may include (but is not limited to) staining for CD45RO (antigen-experienced T cells), CD28, CD27, CD127, CD62L, and CCR7 (T_{CM} cells), CD137 (recent activation), PD-1, Tim-3, LAG3, 2B4, CD160, KLRG1, and CD57 (evidence of negative regulation/senescence). The persistence, function, proliferative

capacity, and phenotype of infused T cells in the T_N and T_{CM} groups will be compared to each other and to their respective cell products at the time of infusion and correlated to clinical responses.

15.K. Evaluation for Long Term Effects of Treatment with Lentivirally Transduced T Cells

1. Testing for Replication-Competent Lentivirus (RCL)

In compliance with FDA Guidance, "Supplemental Guidance on Testing for Replication Competent Retrovirus in Retroviral Vector Based Gene Therapy Products and During Follow-up of Patients in Clinical Trials Using Retroviral Vectors" (As of November 28, 2006, the same rules were stated to apply to lentiviral vectors), every effort will be made to obtain blood samples for testing for replication competent lentivirus (RCL) at the following time points: Pretreatment, at 3, 6 and, 12 months for the first year, and annually thereafter. If all post-treatment assays are negative during the first year, subsequent yearly samples will be archived for future analysis, and analyzed if clinical or scientific events indicate this. Samples will be archived with appropriate safeguards to ensure long-term storage using an efficient system that allows for the prompt linkage and retrieval of the stored samples with the medical records of the patient and the production lot records. If any post-treatment samples are positive, further analysis of the RCL and more extensive patient follow-up will be undertaken, in consultation with the Center for Biologics Evaluation & Research (CBER).

2. Long-Term Follow-Up

Every effort will be made to follow patients for 15 years, in compliance with the FDA Guidance, "Gene Therapy Clinical Trials - Observing Subjects for Delayed Adverse Events" (November 28, 2006). As per this guidance, viruses that have a potential to integrate, including lentiviruses, "present sufficient risk that long-term follow-up (LTFU) observations are necessary to mitigate long-term risks to subjects receiving these vectors." The patients on this study will have follow-up clinical visits on the same time points as testing for RCL occurs (pretreatment, at 3, 6 and, 12 months for the first year, and annually thereafter if possible). At clinical follow-up visits, patients will be examined for clinical evidence suggestive of a potential lentiviral disease, such as cancer, neurologic disorders, and hematologic disorders. Additionally, samples will be collected to determine levels of gene-

modified cells in peripheral blood for 15 years as required by FDA regulations. Suspect clinical symptoms or findings as noted above will trigger performing RCL analysis of archived samples or attempting to obtain additional samples or both, in consultation with CBER. Their local provider may be provided with a blood draw courier kit to enable samples to be returned to Fred Hutch for archival purposes and for analysis such as transferred T cell persistence.

At clinic visits, patients will undergo physical examination and laboratory testing including CBC with differential, comprehensive metabolic panel and assays for levels of gene-modified cells. Details of all exposures to mutagenic agents and other medications will be ascertained and recorded in case histories. Physicians will also be asked to record the emergence of new clinical conditions, including new malignancies, new incidence or exacerbation of a pre-existing neurologic disorder, new incidence or exacerbation of a prior rheumatologic or other autoimmune disorder, and new incidence of a hematologic disorder. Study subjects and health providers will be asked to cooperate in reporting delayed AEs, including unexpected illnesses and hospitalizations. In general, patients will be seen by their doctors in their local area, but the FHCRC in collaboration with SCCA will be available to assist in the LTFU of participants in this clinical trial. If patients die or develop neoplasms, every effort will be made to assay for RCL and lentiviral integration in a biopsy sample of the neoplastic tissue or the pertinent autopsy tissue.

16. MANAGEMENT OF TOXICITIES AND COMPLICATIONS

16.A. Toxicity Grading

Toxicity grading will be evaluated according to the current guidelines in NCI CTCAE v4.0 [118]. The full text of the NCI CTCAE is available online at: http://evs.nci.nih.gov/ftp1/CTCAE/About.html.

16.B. Regimen-related Toxicity

If the patient develops excessive toxicity attributable to one or more component of the regimen, the patient will not receive additional study therapy and therapy with corticosteroids will be given if clinically indicated (please refer to **Section 16.F**). If more than 1 patient develops excessive toxicity, the study stopping rules will apply (see **Section 21.A**).

Excessive toxicity will be considered when a non-pre-existing grade 3 or higher toxicity develops after the start of treatment with the following exceptions:

16.B.1. Expected toxicities attributable to **Cyclophosphamide 300mg/m^2 for 2 days** and considered exceptions to criteria for discontinuation include:

i. Non-life threatening infections (ie, absence of hemodynamic compromise requiring pressor support and non-opportunistic infection).

ii. Transient hematological toxicities: WBC < 1,000 (grade 4 toxicity) for ≤ 4 weeks, lymphocytes < 200 (grade 4 toxicity) for ≤ 4 weeks, platelets < 25 000 (grade 4 toxicity) for ≤ 4 weeks, ANC < 500 (grade 4 toxicity) for ≤ 2 weeks. Of note: G-CSF can be used after the CY infusions at the discretion of the treating physician.

iii. Increased bilirubin and transaminitis (grade 3 or 4 toxicity) for ≤ 2 weeks.

iv. Single laboratory values out of normal that according to the investigator do not have a clinical correlate and resolve to ≤ grade 1 within 7 days with adequate medical management.

16.B.2. Expected toxicities attributable to **T-cell infusions** and considered exceptions to criteria for discontinuation include:

i. Cytokine Release Syndrome (CRS) grade 3 or less, including but not limited to asthenia, flu-like symptoms, myalgia, lymphopenia, and rigors.

ii. Skin rash/Erythroderma (grade 3 toxicity) lasting for < 7 days.

iii. Hypoxemia requiring continuous oxygen, but not mechanical ventilation or intubation < 72 hours.

iv. Fever that resolves within 36 hours of the T-cell infusion.

v. Lymphopenia (lymphocytes < 500) that resolves within 4 weeks to baseline levels (pre-therapy).

vi. Single laboratory values out of normal that according to the investigator do not have a clinical correlate and resolve to ≤ grade 1 within 7 days with adequate medical management.

16.B.3. Expected toxicities attributable to **low-dose s.c. IL-2** considered exceptions to criteria for discontinuation include:

i. Flu-like symptoms (headache, muscle ache, joint ache).

ii. Mild fever.

iii. Redness, pain, and swelling at injection site.

iv. Serious drop in blood pressure requiring intravenous fluids.

v. Cardiac Arrhythmias ≤ grade 2.

vi. Single laboratory values out of normal that according to the investigator do not have a clinical correlate and resolve to ≤ grade 1 within 7 days with adequate medical management.

Patients will have blood cultures drawn for all fevers higher than 38.5 °C according to standard practice guidelines.

16.C Definition and Management/Evaluation of Non-hematologic and Hematologic Toxicities Requiring Treatment Discontinuation

16.C.1. Definition of Dose-Limiting Toxicities

Although unlikely, it is possible that the infusion of $CD8^+$ WT1-specific transduced T cells will result in delayed toxicities related to the recognition of tissues expressing WT1. It is anticipated that the symptoms and signs will occur **within hours to 4 weeks** after completion of either the T-cell infusion or IL-2 administration. Any toxicities requiring treatment discontinuation (see **Section 16.C.2 and 16.C.3**) occurring within 4 weeks of study treatment (potentially due to the T-cell infusion and not attributable to a non-infusion-related cause) will be managed by supportive care and steroids or tocilizumab (at the discretion of the attending and the PI) will be administered as per **Section 16.F.**

16.C.2. Non-hematologic toxicity requiring treatment discontinuation is defined as any grade 3 or 4 non-hematologic toxicity (CTCAE 4) that is deemed to be caused by infusion of the study treatment (ie, not attributable to infection, recurrent/progressive

NSCLC/mesothelioma, toxicity from prior therapies, or any identifiable cause other than CY, T-cell infusion or IL-2 administration) that occurs at any time point after the start of therapy on this study. It is expected that the onset of non-hematologic toxicities will be **within 4 weeks** after completion of the last T-cell infusion although patients will be followed beyond 4 weeks for potential toxicities, as described in **Section 15.C**. Transient high fever may occur due to release of cytokines from T cells stimulated by the recognition of targets, and therefore grade 3 toxicity from fever (> 40°C) lasting no longer than 36 hours will not require treatment discontinuation.

16.C.3. Hematologic toxicity requiring treatment discontinuation (blood/bone marrow CTCAE 4) is defined as any new or recurrent onset of grade 4 hematologic toxicity that occurs at any time point after the first T-cell infusion such as neutropenia (ANC < 500/mm^3) lasting longer than 2 weeks in the context of new bone marrow aplasia (< 5% cellularity), which is attributed to the study treatment (ie, not associated with prior chemotherapy, tumor progression in the bone marrow, infection, or any identifiable cause other than CY, T-cell infusion or IL-2 administration).

16.C.4. Management: If a patient develops new-onset hematologic toxicity such as ANC < 500/mm^3 or platelets < 20 000/mm^3 for 2 consecutive days, which cannot be explained by alternative cause such as known disease progression, bone marrow sample will be obtained.

a. If the bone marrow shows evidence of aplasia, corticosteroids will be started as described in **Section 16.F** and study treatment discontinued. G-CSF may be administered at the discretion of the medical team.
b. If the bone marrow shows no evidence of aplasia and no disease progression or other clear etiology for marrow suppression is found, patients will be observed for 3 more days. If by that time there is no improvement of bone marrow function and no clear etiology for marrow suppression (eg, infection) is detected, corticosteroids will be started as described in **Section 16.F** and study treatment discontinued. G-CSF may be administered at the discretion of the medical team.
c. If bone marrow cannot be obtained and no alternative cause of hematologic toxicity is identified by the third consecutive day of neutropenia or thrombocytopenia,

corticosteroids will be started as described in **Section 16.F** and study treatment discontinued. G-CSF may be administered at the discretion of the medical team.

It is expected that onset of hematologic toxicities will be **within 4 weeks** after completion of study therapy, although patients will be followed beyond 4 weeks for potential toxicities as described in **Section 15.C.**

16.D. Management of Symptoms During T-cell Infusions

Immediate reactions to infusions (ie, defined as those occurring either during the first 24 hours following T-cell infusion) might occur due to release of cytokines from T cells stimulated by the recognition of targets. Mild transient symptoms have been observed with LAK, TIL cell, and antigen-specific T-cell clones (**FHCRC Protocol 2140**).

16.D.1. Milder reactions (ie, < grade 3 CTCAE v.4 or less severe than specified below) would include symptoms such as:

- Fever, chills, fatigue.
- Dyspnea, chest tightness, or myalgia.
- Alteration in vital signs such as:
  - Lowering of blood pressure (BP), but with systolic BP ≥ 90 mmHg, or ≤ 20 mmHg below baseline.
  - Tachycardia, but with heart rate (HR) ≤ 130 or ≤ 30 above baseline.
  - Tachypnea, but with respiration rate (RR) ≤ 32/min or ≤ 10 above baseline.
  - Hypoxemia, but O_2 saturation ≥ 88% on room air, or ≤ 5% fall from baseline.
  - Skin changes such as erythema or urticaria.

Management will include **decreasing the rate of infusion and appropriate supportive care** such as:

- Acetaminophen or Demerol for fever and chills. (All subjects who develop fever or chills should have a blood culture drawn).
- Acetaminophen for headache.
- Diphenhydramine for nausea and vomiting.
- Fluid administration for hypotension.
- Supplemental oxygen for hypoxemia.

16.D.2. More severe reactions would include symptoms such as:

- Hypotension with systolic BP < 90 mmHg and > 20 mmHg below baseline.
- Tachycardia with HR > 130 and > 30 above baseline.
- Tachypnea with RR > 32 and > 10 above baseline.
- Hypoxemia with O_2 saturation of < 88% and > 5% fall from baseline.

Management will include **terminating the infusion and administering supportive medical care**:

- If patient responds to supportive care by normalization of vital signs or resolution of hypoxemia, and the PI or designee deems it safe to continue, the infusion will be restarted at slower rate.
- If the patient does not respond by normalization of vital signs or hypoxemia after supportive care alone, **corticosteroids to ablate the infused T cells can be administered as per Section 16.F**.

16.D.3. Any unexpected severe toxicity (see Section 16.B) occurring in the first 24 hours (due to the T-cell infusion and not attributable to a non-infusion related cause).

Management will be by **supportive medical care and corticosteroids to ablate cells may be administered as per Section 16.F**.

16.E. Management of Severe Cytokine Release Syndromes (or Cytokine Storm)

Blood samples for research tests are planned to be collected in all patients prior to the T-cell infusion, on days1, 3, 7, and 14 after the T-cell infusion, and as indicated in **Section 15.E**. Plasma will also be isolated from each blood sample and stored for future cytokine analysis.

If a patient becomes febrile or develops clinical evidence of a cytokine storm syndrome, we may measure cytokine levels (including IFN-γ, TNF-α, IL-6, IL-2 concentrations), serum ferritin, and markers of tumor lysis syndrome (for example, electrolytes, uric acid, lactate dehydrogenase [LDH]), and evaluate the persistence and the phenotype of the TCR_{C4}-expressing cells at additional time points and as clinically indicated.

Any patient who develops clinical evidence of a cytokine storm syndrome will have a workup

to exclude infection or other causes. Initial treatment should consist of supportive measures as dictated by the clinical and laboratory findings, and may include fluid replacement, antipyretics, oxygen supplementation, and broad-spectrum antibiotics if infection cannot be excluded as a potential etiology for the signs and symptoms. Serious or progressive symptoms and signs may result in the administration of corticosteroids or tocilizumab as described in **Section 16** addressing management of SAEs.

16.F Management of Severe Treatment-related Toxicities

i. If a new and unexpected CTCAE v4.0 grade ≥ 3 toxicity is observed following the T-cell infusion, the patients will receive medical treatment appropriate for the clinical abnormality.

ii. A new and unexpected grade ≥ 3 toxicity that is attributable to the T-cell infusion, is unresponsive to supportive measures, or persists longer than 7 days may be treated with corticosteroids following the suggested dose schedule below (this schedule is adaptable depending on the presence of TCR_{C4}-transduced cells and/or the clinical picture), or tocilizumab (anti-interleukin-6 receptor) after discussion with the PI. *These are general guidelines for the glucocorticoid taper and may have to be adjusted based on each patient's clinical situation*.

Day 1	i.v. Methylprednisolone at 2mg/kg/day if severe p.o. Prednisone at 1mg/kg/day if clinically stable
Day 2	i.v. Methylprednisolone at 2mg/kg/day if severe p.o. Prednisone at 1mg/kg/day if clinically stable
Day 3-4	Prednisone at 1/2mg/kg/day p.o.
Day 5-6	Prednisone at 30mg/day p.o.
Day 7-8	Prednisone at 20mg/day p.o.
Day 9-10	Prednisone at 10mg/day p.o.
Day11-12	Prednisone at 5mg/day p.o.

iii. Patients who require corticosteroids for treatment of severe toxicities will not receive additional treatment on this protocol and will continued to be followed for toxicity until symptoms have resolved. Methylpredisolone (or an equivalent dose of an alternative corticosteroid) can be tapered when symptoms are significantly improving or WT1-specific T cells are less than 0.05% of $CD8^+$ cells in peripheral blood.

iv. It is suggested that patients receiving corticosteroids have daily assessments of peripheral blood for the presence of WT1-specific CTL cells (vβ17^{+} and dual-multimer^{+}) for 5 days and then at least weekly for 4 weeks. Twenty (20) mL of blood will be sent in ACD yellow top tube to the Greenberg Lab (D3-335) for the evaluation of the presence of the infused T cells.

16.G. Concomitant Therapy

16.G.1. Active infections occurring after initiating the study should be treated according to the standard of care.

16.G.2. The following agents are not allowed while on study, and, if needed clinically, will require removal of the patient from the protocol: systemic corticosteroids or tocilizumab (except as outlined for management of toxicity of the transduced CTL), immunotherapy (for example, interleukins, NSCLC vaccines, intravenous immunoglobulin, expanded polyclonal TIL or LAK therapy), pentoxifylline, other small-molecule or chemotherapy cancer treatment, or other investigational agents.

16.H. Off-study Criteria

A patient's participation on the protocol will be terminated for any of the reasons listed below:

1. The participant withdraws consent.
2. Inability to generate transduced T cells that meet release criteria for T-cell therapy.
3. A patient will no longer be eligible to receive additional T-cell infusions if the PI or his designee determines that additional T-cell infusions are not in the best interest of the patient.

The reasons for premature discontinuation must be recorded on the case report form.

17. TARGETED/PLANNED ENROLLMENT

As described in **Section 6** (Study Design), this study plans to treat at least 12 but up to 20 individuals over 3 years with NSCLC or mesothelioma in the advanced metastatic setting. Because the incidence of mesothelioma (up to 4 patients in Arm 1, Stage 2) and patients with NSCLC undergoing neo-adjuvant chemotherapy (up to 4 patients in Arm 2) is low, it is

not known whether these patients can be recruited. The statistics below are for an average of 20 patients.

TARGETED/PLANNED ENROLLMENT:			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	1	3	4
Not Hispanic or Latino	6	10	16
Ethnic Category: Total of All Subjects *	7	13	20
Racial Categories			
American Indian/Alaska Native	0	0	0
Asian	0	1	1
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	1	2	3
White	6	10	16
Racial Categories: Total of All Subjects *	7	13	20

* The "Ethnic Category: Total of All Subjects" must be equal to the "Racial Categories: Total of All Subjects."

All racial groups and ethnicities will be included. **These targeted/planned enrollment numbers are based on relative percentages of race/ethnicity reported for NSCLC cases and on incidence data from the National Cancer Institute, the Centers for Disease Control and Prevention, and the North American Association of Central Cancer Registries and mortality data from the National Center for Health Statistics [14].**

18. GUIDELINES FOR ADVERSE EVENTS REPORTING

18.A. Reporting of Adverse Events (AEs)

All unexpected and serious AEs that may be due to study treatment or intervention must be reported to the FHCRC Institutional Review Office (IRO) per the current reporting requirements.

All grade ≥ 3 CTCAE v4.0 AEs will be recorded from the time of the first study intervention (chemotherapy or T-cell infusion) through 4 weeks after the last T-cell infusion. Beginning 4 weeks after the last T-cell infusion, only study-related serious adverse events (SAE) may be recorded.

All SAEs that are unexpected and related to study treatment will be reported to the FDA on a MedWatch 3500 reporting form that will be submitted to the FDA within 15 calendar days of identifying the event. The reports will include the date and time of onset, severity and duration of the event, the relationship to study treatment, the treatment given, and the eventual outcome.

18.B. Definitions

Definitions associated with reportable events can be found on the FHCRC IRO extranet website. (Relevant FHCRC policies include, but are not limited the documents listed [**Table 3**]. Please also refer to the FHCRC IRO website.)

Table 3. FHCRC IRB policies for reportable events

IRB Policy 2.6	Adverse Events and Other Unanticipated Problems Involving Risks to Subjects or Others	http://extranet.fhcrc.org/EN/sections/iro/irb/ae.html
IRB Policy 1.9	Noncompliance with the Office of the Director's Human Research Protection Program Policy	http://extranet.fhcrc.org/EN/sections/iro/irb/ae.html
IRB Policy 1.1	Reporting Obligations for Principal Investigators	http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html
IRB Policy 2.2	Continuing Review	http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html
IRB Policy 1.13	Investigational New Drugs (IND), Biologics and Investigational Device Exemptions (IDE)	http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html

19. DATA AND SAFETY MONITORING PLAN

19.A. Primary Monitoring

The PI of the study will have primary responsibility for ensuring that the protocol is conducted as approved by the Scientific Review Committee and IRB. The PI will ensure that the monitoring plan is followed, that all data required for oversight of monitoring are accurately reported to the Protocol Office and Data Safety Monitoring Board (DSMB), that

all AEs are reported according to protocol guidelines, and that any adverse reactions reflecting patient safety concerns are appropriately reported.

19.B. Monitoring Plan

The PI, or a co-investigator on the study designated by the PI, will personally review with the Study Nurse the clinical course of all WT1-specific T-cell infusions. The PI or designee will review with the Study Nurse the progress of each patient undergoing therapy as well as the clinical course of all patients who have completed a course of T-cell therapy.

19.C. Monitoring the Progress of the Trial and the Safety of Participants

The FHCRC PI is responsible for monitoring this clinical trial, with oversight by a DSMB, the Protocol and Data Monitoring Committee (PDMC) at the FHCRC, and the FHCRC IRB. This is a Phase I/II study and the assessment of risk is considered above minimal.
The study will be monitored by an external DSMB contracted by Juno Therapeutics. The DSMB will be responsible for safeguarding the interests of trial participants, and assessing the safety and efficacy of the interventions during the trial. This responsibility will be exercised by providing recommendations about stopping or continuing the trial. To contribute to enhancing the integrity of the trial, the DSMB may also formulate recommendations relating to the selection, recruitment and retention of participants and their management; adherence to protocol-specified regimens; and the procedures for data management and quality control.

The DSMB will be advisory to Juno and the PI, who will be responsible for prompt review of the DSMB recommendations to guide decisions regarding continuation or termination of the trial and whether amendments to the protocol or changes in study conduct are required.

The external DSMB is an independent, multidisciplinary group consisting of clinical experts and a statistician who collectively have experience with in the management of patients with NSCLC and mesothelioma and in the conduct and monitoring of clinical trials. The DSMB will meet approximately quarterly to review data.

<u>Statistical Monitoring Guidelines</u>

The DSMB will review all grade 3 or greater toxicities as defined by version 4.0 of NCI

CTCAE and determine whether the study should be prematurely discontinued due to toxicity. The clinical investigators assessing patients will be responsible for evaluations of grade toxicity. Criteria for discontinuing of therapy in an individual patient are described in protocol section titled “Management of Toxicities and Complications”. Criteria for discontinuing the trial is described in section titled “Data and Safety Monitoring”.

The type and grade of toxicities noted during therapy will be summarized for each dose level/infusion. All AEs noted by the investigator will be tabulated according to the affected body system. Changes from baseline in clinical and laboratory parameters should be summarized in a table. Tumor responses will be determined as specified above.

20. RECORDS

The Clinical Research Division at the FHCRC maintains a patient database that allows for the storage and retrieval of specific types of patient data including demographic information, protocol registration information, and data from the treatment course. These data are collected from a wide variety of sources and conform to institutionally established guidelines for coding, collection, key entry, and verification. Each patient will be assigned a unique patient number (UPN) to assure patient confidentiality. Any publication or presentation will refer to patients by this number and not by name. Information about patients enrolled on this protocol that is forwarded to agencies such as the FHCRC IRB, NIH, and FDA will refer to the patients only by their UPN.

Original inpatient and outpatient medical records will be maintained by the medical records departments at the institutions where the patients receive their care. The majority of the care related to this protocol will be received at the SCCA and UW Medical Center. The study nurse or data coordinator will maintain a Case Report Form (CRF) Notebook for each patient treated on this protocol. The CRF notebooks and their contents will be identified by the patient’s initials and UPN only. All supporting documents used to verify the accuracy of the data in the case report forms will be kept separately. Patient research files will be kept in a locked, controlled-access building. At least monthly, the PI or a designated co-investigator will review and cross check the data entered on the case report forms with the source documents.

21. STATISTICAL CONSIDERATIONS

The primary objective of this trial is to assess the safety of adoptive transfer of polyclonal autologous T_N and/or T_{CM} cells transduced with the TCR_{C4} in patients with advanced stage NSCLC and mesothelioma.

21.A. Analysis of Toxicity

Toxicities of combined T_N and/or T_{CM} populations will be taken into consideration as the relative frequencies of T_N and/or T_{CM} cannot be measured in real time. The treatment will be considered to have an acceptable safety profile if the observed toxicity rate is consistent with a true rate that does not exceed 30%, with toxicity defined as described in **Section 17**. Common AEs associated with conventional platinum-based chemotherapy regimens for the systemic treatment of NSCLC include neutropenia, thrombocytopenia, anemia, nausea and vomiting, permanent sensory neuropathy, fatigue, and renal insufficiency. The median rate of grade ≥ 3 toxicities associated with platinum-based chemotherapy regimens exceeds 30% (range 10% to 59%) [119]. Thus, the study will be stopped if there is sufficient evidence to suggest that the true treatment-related toxicity rate exceeds 30%. All patients (Arm 1 Stage 1, Arm 1 Stage 2, and Arm 2) will be analyzed for safety. If there is ever sufficient evidence to suggest that the true probability of treatment-related toxicity as described in **Section 16** is greater than 30%, the trial will be suspended for excess toxicity pending a review by the DSMB. Sufficient evidence will be taken to be an observed toxicity rate whose lower one-sided 80% confidence limit exceeds 30%. Operationally, this will occur if any of the following observed toxicity rates are seen: 2/2, 3/3-5, 4/6-8, 5/9-10, 6/11-13, 7/14-16, 8/17-19, 9/20. If the true probability of toxicity is 20%, then the probability of stopping after 10 or 20 patients is approximately 0.10 and 0.12 respectively; if the true probability is 50%, the probability of stopping is approximately 0.74 and 0.90 respectively (estimated from 5000 simulations).

21.B. Analysis of the Feasibility of infusing TCR_{C4} Transduced T Cells

Feasibility is defined as the ability to generate and infuse the T cells. We anticipate a 4-week period required for T cell generation. We will enroll patients until we are able to grow T cells and infuse them to at least 12 patients. The proportion of patients for which this is

possible and whether only T_N or T_{CM} could be generated will be estimated, and our goal is that at least 80% of patients will be treated. No formal statistical considerations will be used to evaluate this endpoint beyond a simple estimate of the proportion along with its 95% confidence interval.

21.C. Analysis of the Persistence of TCR_{C4} Transduced T Cells

Where possible, we will directly compare within each patient the *in vivo* persistence of cells generated from the T_N subset with cells generated from the T_{CM} subset by HTTCS. All patients enrolled on Arm 1 (Stage 1 and 2) and 2 will be analyzed for this endpoint. Previous data on time to detect persisting transferred T cells among patients who received TCR_{C4}-transduced CTL revealed a median of more than 60 days (see **Section 3.G**). With a minimum of 12 patients treated with both substes, we will have 88% power to detect a statistically significant difference (at the two-sided significance level of .05) in mean T-cell persistence between groups if the true effect size is 1.0 standard deviation units (true difference in means divided by the standard deviation). For example, if the assumed-true persistence is 100 and 60 days for the T_N and T_{CM} groups, respectively, with a standard deviation of 40 days, then this would result in an effect size of 1.0 (100-60)/40. Therefore, it is expected that 12 patients will be sufficient to demonstrate the differences we expect to see. We shall use the one-sample T test to assess this difference, in which the outcome for each patient is the time to disappearance of infused CTL. Patients receiving only one subset, will not be taken in consideration for analysis of persistence of T_N vs T_{CM}.

21.D. Exploratory Analysis of Efficacy of T_N Compared to T_{CM}

We anticipate that in most patients, only cells from the T_{CM} or the T_N subsets will remain detectable after 3 months. For patients who can only receive only 1 subset, we will assess whether the subset they received is detectable at 3 months by HTTCS (limit of detection: 0.001% of CD3). If it is, the data will be taken into consideration. An exploratory objective of this protocol is to assess the potential efficacy of the infused cells and determine which substrate cell (T_N or T_{CM}) is most effective based on the time to progression (TTP) of patients who have persisting T_N cells to that of patients who have persisting T_{CM} cells 3 months after the last infusion. Ultimately, the goal is to select one substrate cell group to move forward for use in future trials. Patients with unresectable NSCLC whose disease is resistant to ≥ first-line treatment (Arm 1 Stage 1 and Arm 1 Stage 2) and patients who are

planned to receive neo-adjuvant chemotherapy in anticipation of a surgical resection (Arm 2) will be enrolled on this study. However, we anticipate that the majority of patients will be enrolled in Arm 1, and this is where a direct comparison will be made between the patients who have persisting T_N or T_{CM} cells.

We plan to enroll enough patients so that at least 12 but preferably 20 will receive TCR_{C4}-transduced T products, of which we expect roughly 9-16 to be enrolled on Arm 1. This number was not derived based on any formal statistical considerations, but rather reflects a compromise between the number that can be enrolled in the 3-year time frame and a protocol duration/size that should provide enough information to gain a preliminary assessment of the potential to achieve more than incremental efficacy. Given the relatively small number of patients, there will be limited power to observe a statistically significant difference in the efficacy endpoint TTP. Rather we will choose the T-cell component which persists after 3 months *in* vivo in patients who have the longest median TTP as the "winner" to take forward for use in subsequent trials. This "winner" will be taken forward only if the observed median TTP is less than 3 months, which is the median TTP observed in patients with NSCLC whose disease is resistant to ≥ first-line therapy. Under the assumption that the risk of progression is constant with respect to time, if the median TTP in one group (for example, patients who have persistent T_N cells) is 3 months and 5 months in the other group, the probability of a longer median observed TTP in the latter group is approximately 0.78; if the true median TTP in the latter group is 6 months, then this probability is approximately 0.86 (probabilities estimated from 500 simulations).

22. ADMINISTRATIVE CONSIDERATIONS

22.A. Institutional Review Board

In accordance with federal regulations (21 CFR 312.66), an IRB that complies with regulations in 21 CFR 56 must review and approve this protocol and the informed consent form prior to initiation of the study.

22.B. Termination of Study

The study will be stopped if any of the following events occur:

- All 20 patients have completed treatment.
- Stopping rules for toxicity have been met. Accrual will be put on hold for discussion with the DSMB regarding a possible change in study design.
- The PI reserves the right to terminate the study at any time. The FDA may also terminate the study.

23. REFERENCES

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APPENDIX A

ECOG / Zubrod Performance Status

0 Fully active, able to carry on all pre-disease performance without restriction

1 Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g. light house work, office work

2 Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours

3 Capable of only limited self-care, confined to bed or chair more than 50% of waking hours

4 Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair

5 Dead

APPENDIX B

Monitoring Schedule*

ARM 1 STAGE 1		Event	History and PE	Renal and Hepatic panel with Mg and LD	CBC	HLA type & WTI marker	Blood Draw for Lab	Radiographic Evaluation
Screening		Evaluation for participation in the study		X	X	X [1]	X [2, 3, 4]	
Leukapheresis or blood draw		Leukapheresis collection			X			
Pre-treatment		Evaluation for T-cell infusion	X	X	X		X[&,4]	X[#]
Week 0	Day 0	Infusion #1(10^9cells/m^2)	X	X	X		X[4,&]	
	Day +1		X	X	X			
	Day +3			X	X		X[&]	
Week +1	Day +7		X	X	X		X[&]	
	Day +11	Cyclophosphamide 300mg/m^2		X	X			
	Day +12	Cyclophosphamide 300mg/m^2		X	X			
Week +2	Day +14	Infusion #2 (10^{10} cells/m^2)	X	X	X		X[&]	
	Day +15		X	X	X		X[&]	
	day +17	IL-2 250,000 IU/m^2 twice daily x 14 days		X	X		X[&]	
Week +3	Day +21			X	X		X[&]	
Week +4	Day +28		X	X	X		X[&]	
Week +6	Day +42		X	X	X		X[&]	X
Week +12	Day +84		X	X	X		X[&]	X
Week +18	Day +126		X	X	X		X[&]	X
Week +24	Day +168		X	X	X		X[&]	X[†]
Beyond 24 weeks			X[†]	X[†]	X[†]		X[&†]	X[†]

* Test results and procedures conducted as per standard of care may be used for eligibility determination. The dates listed on the study calendar above should be respected as much as possible (see **Section 15.E**).

1 WT1 expression from tumor sample at time patient was diagnosed or from subsequent biopsy

2 High resolution HLA-A typing

3 Virology Battery

4 Pregnancy test for women of childbearing potential within 2 weeks of study entry and prior to infusion

[&]60ml in yellow-top (ACD) tubes.

[#] Includes brain evaluation.

[†] At least every 2-3 months and as clinically indicated.

ARM 1 STAGE 2*		Event	History and PE	Renal and Hepatic panel with Mg and LD	CBC	HLA type & WTI marker	Blood Draw for Lab	Radiographic Evaluation
Screening		Evaluation for participation in the study		X	X	X [1]	X [2, 3, 4]	
Leukapheresis		Leukapheresis collection			X			
Pre-treatment		Evaluation for T-cell infusion	X	X	X[5]		X[&4]	X[#]
	Day -3	Cyclophosphamide 300mg/m^2	X	X[6]	X[6]		X[&,6]	
	Day -2	Cyclophosphamide 300mg/m^2		X	X			
Week 0	**Day 0**	Infusion #1 (10^{10} cells/m^2)	X	X	X		X[&]	
	Day +1	IL-2 250,000 IU/m^2 twice daily x 14 days	X	X	X		X[&]	
	Day +3			X	X		X[&]	
Week +1	**Day +7**		X	X	X		X[&]	
Week +2	**Day +14**		X	X	X		X[&]	
Week +3	**Day +21**			X	X		X[&]	
Week +4	**Day +28**		X	X	X		X[&]	
Week +6	**Day +42**		X	X	X		X[&]	X
Week +12	**Day +84**		X	X	X		X[&]	X
Week +18	**Day +126**		X	X	X		X[&]	X
Week +24	**Day +168**		X	X	X		X[&]	X[†]
Beyond 24 weeks			X[†]	X[†]	X[†]		X[†]	X[†]

* Test results and procedures conducted as per standard of care may be used for eligibility determination.The dates listed on the study calendar above should be respected as much as possible (see **Section 15.E**).

1 WT1 expression from tumor sample at time patient was diagnosed or from subsequent biopsy
2 High resolution HLA-A typing
3 Virology Battery
4 Pregnancy test for women of childbearing potential within 2 weeks of study entry and prior to infusion
5 Collect only if no CBC within 15 days of leukapheresis
6 If day -3 falls within 48 hours of pre-treatment lab draw, no need to repeat lab draw at day -3
[&] 60ml in yellow-top (ACD) tubes.
[#] Includes brain evaluation.
[†] Every 2-3 months or as clinically indicated.

ARM 2*		Event	History and PE	Renal and Hepatic Panel	CBC	HLA type & WTI marker	Blood Draw for Lab	Radiographic Evaluation
Screening		Evaluation for participation in the study		X	X	X [1]	X [2, 3, 4]	X[#]
Leukapheresis		Leukapheresis collection			X[5]			
Neo-Adjuvant Chemotherapy								
Pre-treatment (after chemo)		Evaluation for T-cell infusion	X	X[6]	X[6]			
Week 0	**Day 0**	Infusion #1 (10^{10} cells/m^2)	X	X	X		X[&]	
	Day +1	IL-2 250,000 IU/m^2 twice daily x 14 days	X	X				
	Day +3			x	x		X[&]	
Week +1	**Day +7**		X	X	X		X[&]	
Week +2	**Day +14**		X	X	X		X[&]	
Week +3	**Day +21**	Surgery		X	X		X[&]	
Week +4	**Day +28**		X	X	X		X[&]	
Week 6	**Day +42**		X	X	X		X[&]	X
Week +12	**Day +84**		X	X	X		X[&]	X
Week +18	**Day +126**		X	X	X		X[&]	X
Week +24	**Day +168**		X	X	X		X[&]	X[†]
Beyond 24 weeks			X[†]	X[†]	X[†]		X[†]	X[†]

* Test results and procedures conducted as per standard of care may be used for eligibility determination. The dates listed on the study calendar above should be respected as much as possible (see **Section 15.E**).

1 WT1 expression from tumor sample at time patient was diagnosed or from subsequent biopsy

2 High resolution HLA-A typing

3 Virology Battery

4 Pregnancy test for women of childbearing potential within 2 weeks of study entry and prior to infusion

5 Collect only if no CBC within 15 days of leukapheresis

6 If day -3 falls within 48 hours of pre-treatment lab draw, no need to repeat lab draw at day -3

[&] 60ml in yellow-top (ACD) tubes.

[#] Includes brain evaluation.

[†] Every 3 months or as clinically indicated.

APPENDIX C

1. ASSESSMENT OF OVERALL TUMOR BURDEN AND MEASURABLE DISEASE BY RECISIT 1.1 CRITERIA [116].

To assess objective response or future progression, it is necessary to estimate the overall tumor burden at baseline and use this as a comparator for subsequent measurements. Measurable disease is defined by the presence of at least one measurable lesion.

1.1 Measurability of tumor

At baseline, tumor lesions/lymph nodes will be categorized measurable or non-measurable as follows. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

Measurable lesions must be accurately measured in at least one dimension (longest diameter in the plane of the measurement to be recorded) with a minimum size of:

- 10 mm by CT scan - (CT scan slice thickness no greater than 5 mm).
- 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable).
- 20 mm by chest x-ray.
- 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.

All measurements should be recorded in metric notation, using calipers if clinically assessed.

Special considerations regarding lesion measurability:

Bone lesions:

- Bone scan, PET scan or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.
- Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be considered as measurable lesions if the soft tissue component meets the definition of measurability described above.
- Blastic bone lesions are non-measurable.

Cystic lesions:

- Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

- 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions. □

Lesions with prior local treatment:

- Tumor lesions situated in a previously irradiated area, or in an area subjected to other loco- regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion.

Non-measurable lesions are all other lesions, including small lesions (longest diameter < 10mm or pathological lymph nodes with ≥ 10 to < 15 mm short axis), as well as non-measurable lesions. Lesions considered non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

1.2 Method of Assessment

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 should always be performed rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical examination.

CT, MRI: CT is the best currently available and reproducible method to measure lesions selected for response assessment. Measurability of lesions on CT scan is based on the assumption that CT slice thickness is 5 mm or less. When CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness.

Chest X-ray: Chest CT is preferred over chest x-ray, particularly when progression is an important endpoint, since CT is more sensitive than x-ray, particularly in identifying new lesions. However, lesions on chest x-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial and ≥ 10 mm diameter as assessed using calipers. For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is suggested. As noted above, when lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may be reviewed at the end of the study.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised.

Endoscopy, laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised.

Tumor markers: Tumor markers alone cannot be used to assess objective tumor response.

2. BASELINE DOCUMENTATION OF 'TARGET' AND 'NON-TARGET' LESIONS

Target lesions: When more than one measurable lesion is present at baseline all lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions and will be 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, and should lend themselves to reproducible repeated measurements.

Lymph nodes merit special mention since they are normal anatomical structures which may be visible by imaging even if not involved by tumor. Pathological nodes which are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of □ 15 mm by CT scan. Only the short axis of these nodes will contribute to the baseline sum. All other pathological nodes (those with short axis □ 10 mm but < 15 mm) should be considered non-target lesions. Nodes that have a short axis < 10 mm are considered non-pathological and should not be recorded or followed.

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. 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 pathological lymph nodes should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as 'present', 'absent', or 'unequivocal progression'. In addition, it is possible to record multiple non-target lesions involving the same organ as a single item on the case record form (eg, 'multiple enlarged pelvic lymph nodes' or 'multiple liver metastases').

3. TUMOR RESPONSE EVALUATION AND RESPONSE CRITERIA (EFFICACY ASSESSMENT).

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 diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of 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 from the baseline study to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

Special notes on the assessment of target lesions:

- **Lymph nodes**: Lymph nodes identified as target lesions should always have the actual short axis measurement recorded and should be measured in the same anatomical plane as the baseline examination, even if the nodes regress to below 10 mm on study. This means that when lymph nodes are included as target lesions, the 'sum' of lesions may not be zero even if complete response criteria are met, since a normal lymph node is defined as having a short axis of < 10 mm.

- **Target lesions that become 'too small to measure'**: All lesions (nodal and non-nodal) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (eg, 2 mm). If the radiologist is able to provide an actual measure, that should be recorded, even if it is below 5 mm. However, when such a lesion becomes difficult to assign an exact measure to then:

  i. if it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0 mm.
  ii. if the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned (note: in case of a lymph node believed to be present and faintly seen but too small to measure, a default value of 5 mm should be assigned in this circumstance as well). This default value is derived from the 5 mm CT slice thickness (but should not be changed with varying CT slice thickness).

Lesions that split or coalesce on treatment: When non-nodal lesions 'fragment', the longest diameters of the fragmented portions should be added together to calculate the target lesion sum. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in obtaining maximal diameter measurements of each individual lesion. If the lesions have coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximal longest diameter for the 'coalesced lesion'.

3.2 Evaluation of non-target lesions

While some non-target lesions may actually be measurable, they need not be measured and instead should be assessed only qualitatively at the time points specified in the protocol.

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).

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): Unequivocal progression of existing non-target lesions. (Note: the appearance of one or more new lesions is also considered progression).

The concept of progression of non-target disease requires additional explanation as follows:

I. **When the patient also has measurable disease**: To achieve 'unequivocal progression' on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest 'increase' in the size of one or more non-target lesions is usually not sufficient to qualify for unequivocal progression status.

II. **When the patient has only non-measurable disease:** To achieve 'unequivocal progression' on the basis of the non-target disease, there must be an overall level of substantial worsening such that the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest 'increase' in the size of one or more non-target lesions is usually not sufficient to qualify for unequivocal progression status. Because worsening in non-target disease cannot be easily quantified (by definition: if all lesions are non-measurable) a useful test that can be applied when assessing patients for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease: ie, an increase in tumor burden representing an additional 73% increase in 'volume' (which is equivalent to a 20% increase diameter in a measurable lesion). Examples include an increase in a pleural effusion from 'trace' to 'large', an increase in lymphangitic disease from localized to widespread, or may be described in protocols as 'sufficient to require a change in therapy'. If 'unequivocal progression' is seen, the patient should be considered to have had overall PD at that point.

3.3 New lesions

The appearance of new malignant lesions denotes disease progression. The finding of a new lesion should be unequivocal: ie, not attributable to differences in scanning technique, change in imaging modality or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). This is particularly important when the patient's baseline lesions show partial or complete response. For example, necrosis of a liver lesion may be reported on a CT scan report as a 'new' cystic lesion, which it is not.

A lesion identified on a follow-up study in an anatomical location that was not scanned at baseline is considered a new lesion and will indicate disease progression. An example of this is the patient who has visceral disease at baseline and while on study has a CT or MRI brain ordered which reveals metastases. The patient's brain metastases are considered to constitute PD even if he/she did not have brain imaging at baseline.

If a new lesion is equivocal, for example because of its small size, continued therapy and follow-up evaluation will clarify if it represents new disease. If repeat scans confirm that

there is a new lesion, then progression should be declared using the date of the initial scan.

3.4 Tumor markers

Tumor markers alone cannot be used to assess objective tumor responses. If markers are initially above the upper normal limit; however, they must normalize in order for a patient to be considered as having attained a complete response.

4. EVALUATION OF BEST OVERALL RESPONSE

4.1 Time point response

A response assessment for each patient should occur at each time point specified in the protocol. Best overall responses of patients who have received naïve polyclonal $CD8^+$ T cells will be compared to patients who have received central memory polyclonal $CD8^+$ T cells on Arm 1 (Stage 1 and Stage 2 combined) only.

Table 1 provides a summary of the overall response status calculation at each time point.

Appendix Table 1: Summary of the Overall Response Status Calculation [Time point response: patients with target (+/-) non-target disease]

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

4.2 Missing assessments and inevaluable designation

When no imaging/measurement is done at all at a particular time point, the patient is not evaluable (NE) at that time point. If only a subset of lesion measurements are made at an assessment, usually the case is also considered NE at that time point, unless a convincing argument can be made that the contribution of the individual missing lesion(s) would not change the assigned time point response. This would be most likely to happen in the case of PD.

4.3 Special notes on response assessment

When nodal disease is included in the sum of target lesions and the nodes decrease to 'normal' size (< 10 mm), they may still have a measurement reported on scans. This measurement should be recorded even though the nodes are normal in order not to overstate progression should it be based on increase in size of the nodes. As noted earlier, this means that patients with CR may not have a total sum of 'zero' on the case report form (CRF).

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 objective progression even after discontinuation of treatment. Symptomatic deterioration is not a descriptor of an objective response: it is a reason for stopping study therapy.

For equivocal findings of progression (eg. very small and uncertain new lesions; cystic changes or necrosis in existing lesions), treatment may continue until the next scheduled assessment. If at the next scheduled assessment, progression is confirmed, the date of progression should be the earlier date when progression was suspected.

5. ADDITIONAL CONSIDERATIONS

5.1 Duration of response

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

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

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest sum on study (if the baseline sum is the smallest, this is the reference for calculation of PD).

5.2 Lesions that disappear and reappear

If a lesion disappears and reappears at a subsequent time point it should continue to be measured. However, the patient's response at the point in time when the lesion reappears will depend upon the status of his/her other lesions. For example, if the patient's tumor had

reached a CR status and the lesion reappeared, then the patient would be considered PD at the time of reappearance. In contrast, if the tumor status was a PR or SD and one lesion which had disappeared then reappears, its maximal diameter should be added to the sum of the remaining lesions for a calculated response: in other words, the reappearance of an apparently 'disappeared' single lesion amongst many which remain is not in itself enough to qualify for PD: that requires the sum of all lesions to meet the PD criteria. The rationale for such a categorization is based upon the realization that most lesions do not actually 'disappear' but are not visualized because they are beyond the resolving power of the imaging modality employed.

5.3 Use of 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. Confirmatory CT is recommended.

No FDG-PET at baseline and a positive FDG-PET at follow-up:

I. If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD.

II. 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 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. Reference:

6. ASSESSMENT OF OVERALL TUMOR BURDEN AND MEASURABLE DISEASE BY RECIST 1.1 mesothelioma modified [117].

Although RECIST 1.1 criteria will be used as a benchmark to assess tumor responses, RECIST 1.1 mesothelioma modified will be assessed in parallel and compared. In contrast to RECIST and model World Health Organization (mWHO) criteria, RECIST 1.1 mesothelioma modified takes into consideration the particularities of mesothelioma and the pleural thickening associated with it. Details of RECIST 1.1 mesothelioma modified can be found in "A practical guide of the Southwest Oncology Group (SWOG) to measure malignant pleural mesothelioma (MPM) tumors by RECIST and modified RECIST criteria"[120] and this protocol will follow the standards described in this article.