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TITLE OF PROTOCOL: PHASE I/II STUDY OF AUTOLOGOUS (CENTRAL MEMORY/NAÏVE) CD8$^+$ T CELLS THAT HAVE BEEN TRANSDUCED TO EXPRESS A WT1-SPECIFIC T CELL RECEPTOR FOR TREATMENT OF AML

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INTRODUCTION

Acute myeloid leukemia (AML) is the most common acute leukemia in adults, accounting for approximately 80 percent of cases in this group. Despite advances in diagnosis and therapy, the outcome of AML patients remains poor, with less than 30% 5-year overall survival (OS) on average.[1,2] Among patients who initially achieve a complete remission with induction therapy, the vast majority will ultimately relapse and die of disease-related complications.[3] Allogeneic HCT dramatically reduces rates of disease relapse, and thus offers a potentially curative approach for many high-risk patients, yielding ~30-50% prolonged disease free survival (DFS).[4,5] However, many high-risk patients, due to age, comorbidity or lack of an available donor, are not candidates for HCT and, thus, alternative consolidative approaches are needed.

WT1 is expressed in cells of the developing genitourinary system as well as in early hematopoietic or CD34+ progenitor cells, but notably absent in mature leukocytes [6]. WT1-specific cytotoxic T lymphocytes (CTL) in vitro and in vivo can recognize and kill leukemia cells that express abnormally high levels of WT1, without affecting normal cells that express lower levels of WT1.[7-13] Our lab has developed methods to isolate and expand high avidity CD8+ CTL clones specific for WT1 from normal donors.[14] In a clinical trial for patients with high-risk leukemias undergoing allogeneic hematopoietic cell transplantation (HCT), eleven patients were infused with WT1-specific $CD8^+$ T cell clones generated from each patient's matched donor.[15] 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 to the identification and isolation of a high-affinity WT1-specific T cell receptor (TCR), denoted TCR_{C4}, for use in a protocol for post-transplant treatment of leukemia patients.

To date, 25 AML patients have been treated with cells expressing the TCR_{C4} after HCT; the preliminary data suggest this approach is safe, as patients have not experienced significant toxicities, including no evidence of damage to organs expressing physiologic levels of WT1 despite the long-term persistence of the T cells in most patients.[15] Furthermore, we have encouraging data supporting the potential efficacy of WT1-specific $CD8^+$ T cells in preventing post-transplant relapse, in that we have observed a 92% relapse-free survival in 13 high-risk patients at a median of 23 months follow-up post-transplant. By comparison, patients who receive

myeloablative transplants from HLA-identical siblings demonstrate a relapse rate of ~25% at three months and ~40% at 1 year post-transplant.[16-20]

The proposed study is a Phase I/II trial aimed at treating up to 35 patients with AML who achieve morphologic remission following induction therapy, and who are determined to be at extremely high risk for relapse, but who either are not candidates for, or elect against, HCT following standard consolidation chemotherapy. Enrolled patients will receive adoptively transferred autologous T cells genetically modified to express TCR_{C4}. We will assess the safety and feasibility of this approach, the ability of the cells to persist and localize to the bone marrow, and the potential clinical efficacy of WT1-specific T cells in clearing minimal residual disease (MRD), reducing relapse, and improving survival outcomes. We predict that both initial and long-term clinical responses will correlate with the persistence of a functional population of antigen-specific CTL. Patients in morphologic CR who have MRD detectable by flow cytometry, or who have incomplete recovery of peripheral blood counts have an estimated rate of relapse as high as 90% by 1 year, [21-23] and therefore represent an appropriate high-risk population to be considered for inclusion in the study. A contemporaneous cohort of high-risk patients who do not receive the study intervention, but who similarly are not candidates for HCT, will be followed prospectively to allow for an assessment of efficacy based on comparisons of relapse, DFS and OS. The expected high rate of relapse by 1 year, without HCT, suggests that we will be able to draw meaningful conclusions regarding the efficacy of our study intervention within only a few years of treating patients.[20,22-28]

Although studies have demonstrated that transferred T cells of the central memory (T_{CM}) subtype can provide enhanced protective immunity *in vivo,*[29] increased clonal expansion and anti-tumor activity of transferred murine $CD8^+$ CTL have also been observed when effector cells were derived from the naïve pool (T_N) as compared to the T_{CM} pool.[30] Thus, the nature of the T cell that would be the most effective source of CTL for adoptive tumor therapy remains controversial. In this study, we plan to evaluate the feasibility of generating and co-infusing WT1-specific $CD8^+$ T cells derived from the T_N and T_{CM} subset, directly compare their persistence, localization to the bone marrow, and potential function of CTL against residual leukemia. Additionally, distinct memory subpopulations may have distinct advantages because of their specificity, such as EBV-specific memory T cells, which are generally enriched for T cells of the T_{CM} phenotype and have the potential advantage of being intermittently triggered and expanded *in vivo* from transient viral

reactivation. Thus, we will also assess the feasibility of generating and infusing EBV-specific T cells that have been rendered WT1-specific.

BACKGROUND

3.A. Prognosis and current treatment options for AML

As noted above, AML is the most common acute leukemia in adults, accounting for approximately 80 percent of cases in this group. Despite advances in diagnosis and therapy, the outcome of AML patients remains poor, with average 5-year overall survival (OS) of ≤30%.[1,2,20,31] The median age at diagnosis is >65 years, and outcomes are even poorer for older patients, with 5-year OS of only 5-10%.[32] Survival outcomes are generally better in pediatric AML patients, overall, although those with poor-risk features continue to demonstrate high rates of relapse and poor survival. [33,34] Patient age, cytogenetic and molecular classification, and performance status remain the most well-established predictors of response to therapy.[35-37] Thus, younger patients and those with favorable cytogenetics are most likely to achieve a morphologic CR, defined by less than 5% blasts in the bone marrow, absence of extramedullary disease, and recovery of neutrophil counts to ≥1,000/mm^3 and platelet counts to ≥100,000/mm^3.[38] However, for patients who do initially respond well to chemotherapy by achieving CR, relapse remains a persistent problem. For those who receive consolidation chemotherapy alone as post-remission therapy, the 5-year cumulative incidence of relapse ranges from 35% in those with favorable risk cytogenetics to 80% in those with unfavorable cytogenetics.[3]

As a result of the graft versus leukemia effect mediated by alloreactive T cells,[39,40] allogeneic HCT dramatically reduces rates of disease relapse, and thus offers a potentially curative approach for many patients, albeit at the expense of considerable morbidity and transplant-related mortality.[25,26,28,41] Allogeneic HCT after an initial CR yields prolonged DFS in roughly 30-50% of AML patients.[5,42] In a meta-analysis of prospective trials including more than 6000 younger patients (age < 60 years) receiving HCT in first complete remission, a statistically significant improvement in OS has been demonstrated in unfavorable-risk patients (HR 0.69) and intermediate-risk patients (HR 0.76).[5] Several retrospective analyses suggest that the survival benefit associated with HCT, albeit with reduced-intensity conditioning, likely extends to older

patients, as well.[43-45] Thus, it is generally accepted that HCT be considered in medically fit, high-risk patients in first CR who have a suitable donor.

Unfortunately, even with the development of reduced-intensity regimens and alternative donor options, many patients, particularly older patients with significant comorbidities, remain ineligible for HCT.[3] At our institution, it was reported that of 212 newly diagnosed patients who were 18-75 years of age, 116 (67%) underwent HCT.[46] The remaining patients did not receive HCT because of age, comorbidity, or lack of a suitable donor. In older patients, who represent the majority of those not undergoing HCT, DFS with chemotherapy alone as post-remission therapy is in the range of 20-35% at 2 years, [43-45] and patients with selected high risk features have an even poorer DFS of approximately 10% at 1 year without HCT.[21-23,47] Therefore, alternative strategies are needed for patients not receiving a HCT. The development of strategies for targeting tumor-associated antigens that bypass the patient's tolerance mechanisms may provide a potentially effective therapeutic approach. Adoptively transferred autologous T cells genetically modified to express a high affinity TCR that recognizes an antigen expressed by AML cells represents one such strategy.

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

The WT1 gene, located at 11p13q, encodes a 52-54 kDa protein transcription factor containing four C-terminal DNA-binding zinc fingers.[48] WT1 plays an important role in cellular proliferation and differentiation in normal hematopoiesis. *In vitro* and *in vivo* studies in mice have shown that WT1 has multiple functions, including gene transactivation, gene repression and RNA binding.[49]. It is involved in the regulation of genes encoding growth factors (e.g. TGFβ, CSF-1), growth factor receptors (e.g. insulin-R, IGF-1R, EGFR), transcription factors (e.g. EGR, c-Myc, Pax2, Dax-1, and Sry) and anti-apoptotic molecules (bcl-2 and bcl-xl).[48,50,51] During embryogenesis, WT1 plays a critical role in the development of the genitourinary tract, spleen, and mesothelial structures,[52] but WT1 expression after birth is limited to very low levels primarily in kidney podocytes, testes sertoli cells, ovarian granulosa cells, mesothelial cells of the lung, the uterus and fallopian tubes, and $CD34^+$ hematopoietic progenitor cells.[52-55]

The WT1 gene was originally identified as a tumor suppressor gene in pediatric kidney cancers, but it subsequently became evident that the role of WT1 is more context dependent and has a

broader role in the oncogenesis of hematological malignancies, including AML, and solid tumors, in which overexpression promotes oncogenesis rather than suppresses tumor formation. Overexpression of the WT1 gene is detected by reverse transcription polymerase chain reaction (RT-PCR) in many leukemias, including ~80-100% cases of adult and pediatric AML, myelodysplastic syndrome (MDS) and chronic myeloid leukemia (CML), and in solid tumors including breast, ovarian, and pancreatic and lung cancers.[56-58] WT1 is also mutated in approximately 10% of AMLs, which can lead to overexpression.[59,60] High levels of WT1 expression have been correlated with a poor prognosis in leukemia patients.[61,62] Because WT1 promotes proliferation and oncogenicity, loss of expression is disadvantageous for tumor that overexpress the gene, leading to growth arrest and apoptosis, which reduces the risk of outgrowth of antigen-loss variants if targeting this protein immunologically.[63]

The ubiquity of WT1 in leukemia has led to the testing of vaccines aimed at employing the host immune system to mount a T cell response to this antigen. However, the development of effective cancer vaccines has progressed poorly, and a comprehensive review of cancer vaccine trials involving 440 patients disappointingly revealed an objective clinical response rate of <3%, highlighting the substantive obstacles to generating an effective anti-tumor immune response in cancer patients.[64,65] Similarly, although vaccines targeting WT1 have produced clear antitumor responses in some patients, including complete remissions in patients with AML, without toxicity to normal tissues, most patients failed to benefit clinically, reflecting in part the induction of responses of low magnitude and/or low avidity due to the restricted repertoire to this self-protein and the limited immunogenicity of current vaccine regimens.[66-71]

Adoptive T cell therapy with $CD8^+$ CTL specific for antigens expressed by AML cells, but absent or expressed at only low levels by normal hematopoietic progenitor cells, has the potential to selectively eliminate leukemia cells. 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}) 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 and expressed in all described WT1 isoforms. As no coding mutations of this epitope have been reported, it is considered a promising immunotherapeutic target.[72]

3.C. Rationale for treating AML patients in CR with minimal residual disease or incomplete recovery of peripheral blood counts, irrespective of cytogenetic risk

It has become increasingly evident that the quality of remission achieved with first induction is a major predictor of outcomes, and may be more important prognostically than are conventional pre-treatment patient and disease characteristics such as age or cytogenetic classification. Multi-parameter flow cytometry (MPFC) techniques are able to detect aberrant immunophenotypes in as low as one leukemic cell in as many as 100,000 events (0.001%), and have been widely integrated into standard clinical practice for detection of minimal residual disease (MRD). Between 60-90% of adult and pediatric patients found to be MRD-positive at the time of CR will relapse within 1-2 years, compared to roughly 25-40% of those who are MRD-negative.[21,23,24,47,73] In the United Kingdom MRC AML12 trial, 3-year event-free survival of pediatric patients with MRD ≥0.5% at end of induction was only 15%, compared to 85% in MRD-negative patients.[74] The association of MRD and relapse remains statistically significant even after correction for other factors, including age and cytogenetics, and has been shown to be predictive even when MRD is present following induction and prior to consolidation chemotherapy. However, these reported rates of relapse with MRD are often confounded by the inclusion of patients who undergo allogeneic HCT. In a Seattle cohort of patients, ages 18-80, and found to be MRD-positive at the time of CR, the observed rate of relapse was between 80-90% at 1 year in those who did not undergo HCT. [22,23,47] Similarly, an Italian group reported that 80% of MRD-positive patients who did not undergo HCT relapsed at a median of only 5 months. [21]

In addition, recent evidence suggests that patients who attain a morphologic CR but with incomplete recovery of neutrophil counts - less than 1,000/mm^3 (CRi) or platelet counts less than 100,000/mm^3 (CRp) - have a substantially increased risk of relapse and markedly poorer survival than those with CR and adequate recovery of peripheral blood counts, independent of other prognostic variables.[20,47,75] Indeed, CRi has been designated by an International Working Group as a separate category of response in clinical trials. [38] In pediatric patients, a platelet threshold of < 80,000/µl will be used to define CRp, as per consensus guidelines for evaluating pediatric response to therapy.[76] In the above Seattle cohort, of which approximately half underwent an allogeneic HCT, the risk of relapse increased from 20% to 80% at 3 years in those with CRi/CRp compared to those with CR and good recovery of peripheral counts.[22] When analysis was restricted to only those patients who did not undergo HCT, the negative impact of CRi/CRp was

even more profound, with a 90% rate of relapse at 1 year. [47] As shown in Figure 1, below, the expected rate of relapse for patients with *either* CRi/CRp or MRD not undergoing HCT was approximately 90% at 1 year (**Figure 1,** below).

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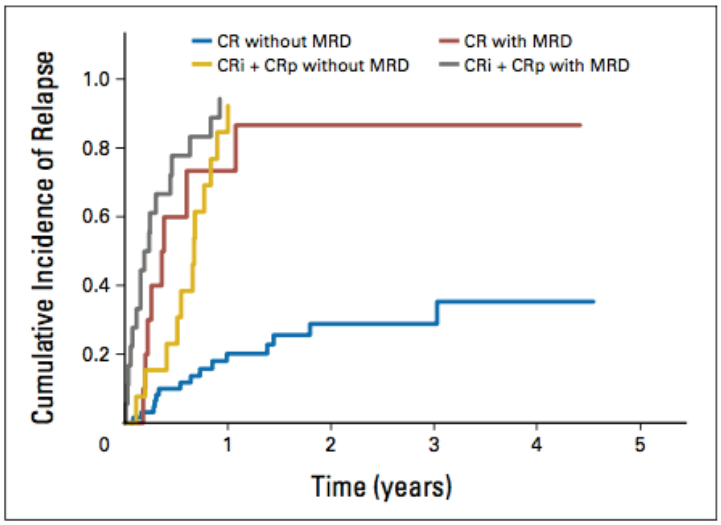
Figure 1. Cumulative incidence of relapse in patients not undergoing HCT, stratified by presence or absence of MRD as determined by flow cytometry and by peripheral blood count recovery (CR versus CRi/CRp)



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 High Risk AML

Adoptive transfer of donor-derived *ex vivo*-expanded WT1-specific CD8$^+$ CTLs in patients with AML can potentially bypass the limitations encountered during vaccination by increasing the number and quality of T cells targeting WT1. Our lab has developed methods to generate high avidity CD8$^+$ T cell responses specific for WT1 by primary *in vitro* sensitization of T_N cells from healthy donors.[14] 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.[77] Although we successfully generated WT1-specific CD8$^+$ CTL clones from >85% of donors, this effort was very time consuming, commonly requiring 12-16 weeks, which can be problematic for leukemia patients who may need therapy more promptly, and the avidity of the T cells generated from each donor for the WT1 epitope presented in MHC class I was variable, with the lower avidity responses exhibiting less reactivity *in vitro*, which likely limited *in vivo* anti-tumor activity in those patients.

Isolating and transferring a high affinity TCR into primary T cells has the potential to efficiently impart specificity as well as high avidity for a cell expressing the desired target antigen, and

makes it possible to rapidly and reproducibly generate tumor-reactive T cells for therapy. Genes encoding the α and β chains of a TCR specific for a candidate tumor antigen have been shown to be adequately expressed in T cells and to mediate antitumor effects in patients.[78]

One potential limitation of TCR-transduced T cells is that the introduced TCR chains can inappropriately pair with the endogenous TCR chains, producing TCRs with unpredicted and potentially auto-reactive specificities, as well as reducing expression of the correctly paired chains and decreasing the avidity compared to the original "donor" T cell. However, our lab demonstrated that introducing a point mutation into each of the TCR chains, to insert a cysteine into the Cα and the Cβ domains, created an interchain disulfide-bond that resulted in preferential pairing of the introduced α and β chains, reduced mismatching with endogenous TCR chains, and increased expression compared to the endogenous TCR chains. Transduced T cells had higher avidity than cells transduced to express chains without the mutation.[79]

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).[80] Modifying the TCRα and β gene sequences to encode each amino acid with the optimal codon for human gene expression, as well as eliminating mRNA instability motifs and cryptic splice sites, has significantly enhanced expression of introduced TCRα and β genes.[81]

The WT1-specific TCR being used in this protocol (TCR_{C4}) was selected after screening >1000 T cell clones isolated from the repertoires of >70 normal healthy individuals for avidity, as reflected by increased WT1-specific cytolytic activity for targets expressing decreasing levels of WT1 and by enhanced binding of peptide/MHC tetramers. To minimize the potential risk of on-target off-tissue toxicity (i.e., recognition of the few normal tissues that express physiologic low levels of WT1, 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 TCR_{C4} expressing T cells did not mediate an autoimmune process in the periphery of the donor.

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TCR_{C4} 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 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,[82,83] 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.[79]

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 gene therapy trials in which five patients developed acute lymphoblastic leukemia, 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 actively transcribed, which may be particularly problematic in transduced HSC 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.[84]

By contrast, the risks of insertional oncogenesis or other vector-related cellular toxicities are extremely low when targeting peripheral blood T cells that lack the same self-renewal capacity of HSC, with no significant toxicities reported to date in any clinical trial. As described below, this risk can be further decreased with the use of a third generation self-inactivating (SIN) lentivirus (LV), 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.[85]

Insertional oncogenesis was not observed in the long-term results from three clinical trials evaluating γ-RV-engineered T cells for patients with HIV.[86] The transduced cells persisted long term, in some cases >10 years, and clinical monitoring of the patients at yearly intervals for cumulatively >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.[87,88] The MSGV1-based γ-RV was also used to transduce autologous T cells to express an NY-ESO-1-specific TCR that was infused in 17 patients with metastatic synovial sarcoma or melanoma[89] and to transduce cells to express a carcinoembryonic antigen (CEA)-specific TCR in T cells that were infused into three patients. Again, no insertional mutagenesis events were identified.

Transcriptionally active enhancer/promoter elements may influence expression of cellular genes at a distance from the insertion site, independently of the vector type used to introduce the gene (RV vs LV; LTR-based or SIN).[90] However, γ-RVs have a predilection toward integrating in the immediate proximity of transcription start sites and deoxyribonuclease I hypersensitivity sites,[91] increasing the probability that the viral LTR transcriptional enhancer will interfere with gene regulation and potentially activate cancer-causing genes.[92] In contrast, LVs commonly insert into introns, and thus are more likely to integrate further away from the transcription start sites of active transcription units, making them less likely to induce transcriptional activation.[93] *In vivo* genotoxicity assays based on the transplantation of transduced tumor-prone *Cdkn2a-/-* murine hematopoietic progenitor cells have also 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.[94]

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 AML. 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 per current guidelines, which are outlined in **Section 15** and **Section 16**. Stopping rules will also be applied, as described in **Section 23**.

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

Establishing a persistent functional population of antigen-specific CTL after adoptive transfer will likely be necessary to eliminate AML cells and prevent recurrence. Previous trials have often been limited by the inability of transferred T cells to expand and persist *post-* 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 are derived,[95,96] and we can choose the T cell type used for adoptive cell therapy.

Conventional $CD8^+$ T cells can be divided into naïve and antigen-experienced, memory T cell subtypes (T_N, T_M). T_M cells can be further divided into T_{CM} and effector memory T cell (T_{EM}) subsets, which have distinct transcriptional programs associated with defined characteristics, including homing, phenotype, and function.[97] When T_{EM} cells are stimulated *in vitro*, they differentiate largely into short-lived effector cells that kill targets, but generally have limited proliferative capacity and fail to persist for long periods of time after transfer.[95,97-99]

Studies of transferred purified $CD8^+$ T cell subsets in a murine virus infection model revealed that transferred T_{CM} provide enhanced protective immunity from *in vivo* challenge compared to T_{EM} [96]. Although T_{CM} cells expand and also differentiate into effector cells in response to *in vitro* stimulation, adoptive transfer studies in non-human primates revealed that T_{CM}–derived effectors had been imprinted and retained some of the beneficial properties of their parent T_{CM} cell, in particular the capacity for self-renewal, which translates into improved *in vivo* persistence and response to antigen challenge.[95]

Increased persistence after transfer was also observed with murine $CD8^+$ CTLs derived from the naïve pool when these cells were primed in the presence of the γ_c-chain cytokine, Interleukin-21 (IL-21).[100,101] In both murine and human studies, adding IL-21 with Interleukin-15 to T cell cultures during the *in vitro* priming of antigen-specific $CD8^+$ T cells has been shown to not only induce greater expansion and to prevent apoptosis of the cells responding to antigen stimulation, but to also lead to *in vitro* generation of $CD8^+$ T cells that are $CD28^{hi}$, reflecting a less terminally differentiated phenotype.[101,102] We have also shown in humans that WT1-reactive CTL clones derived from naïve donors and primed in the initial presence of IL-21 expressed significantly higher levels of CD27, CD28, and CD127 prior to infusions than cells not exposed to IL-21, which

is again consistent with a less differentiated phenotype.[77] 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 are a valid therapy alternative to T_{CM}-derived CTL, and may have some potential advantages as a cell source.

In this study, we will evaluate the feasibility of generating genetically-engineered WT1-specific $CD8^+$ CTL derived from T_N and T_{CM} subsets, and compare their *in vivo* persistence, localization to the bone marrow, and function in eliminating leukemia cells. Patients enrolled in this trial will initially receive a mix of sorted $CD8^+$ T cells from the T_N subset and the T_{CM} subsets, both of which will have been subsequently transduced with 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)[103,104,105]. This can then serve as a 'bar code' of cells derived from either the T_N or T_{CM} populations.[106] The trial will evaluate and compare the *in vivo* behavior of these two T cell populations after they are infused, determining respective frequencies, persistence, function, and localization to the bone marrow. These results will be correlated with the clinical outcomes of the patients.

3.G. Rationale for Comparison of TCR_{C4}-transduced cells derived from T_N- and T_{CM}-subsets with those derived from EBV-specific cells

Our clinical experience with donor-derived TCR_{C4}-transduced $CD8^+$ cells in a high-risk leukemia population after allogeneic transplant consists of cells derived from virus-specific (EBV- and CMV-specific) T cells on **FHCRC Protocol 2498.** In this trial, *in vivo* expansion and persistence was greatly increased in patients who received cells derived from donor EBV-specific compared to cells derived from CMV-specific cells. Transduced EBV-specific cells persisted at higher frequencies (sometimes up to 50% of $CD8^+$ T cells) for long periods of time (>1 year). Analysis of EBV- and CMV-specific populations in the donors revealed that surface markers associated with long lived memory (CD28, CD127, CD62L and CCR7) were expressed at significantly higher levels on EBV-specific compared to CMV-specific cells, consistent with a less-differentiated (more central memory-like) phenotype (**Figure 2**, unpublished data). Comparatively, markers associated

with exhaustion/activation (TIM3, 2B4, and KLRG1) were decreased on EBV-specific cells (**Figure 3**, unpublished data) compared to the CMV-specific subset. This suggests EBV-specific cells, because of their specificity and/or phenotypic state, may also have characteristics that render them a favorable $CD8^+$ T cell subset to transduce with TCR_{C4} and subsequently transfer.

Figure 2. Differential expression of memory markers on donor EBV- and CMV-specific T-cells

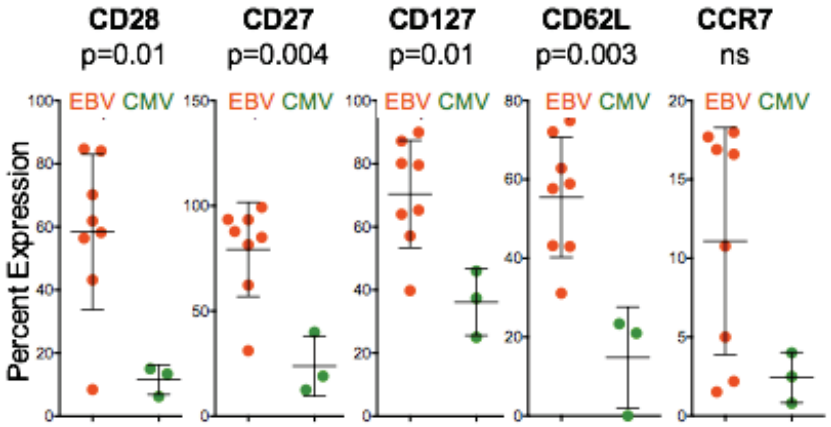
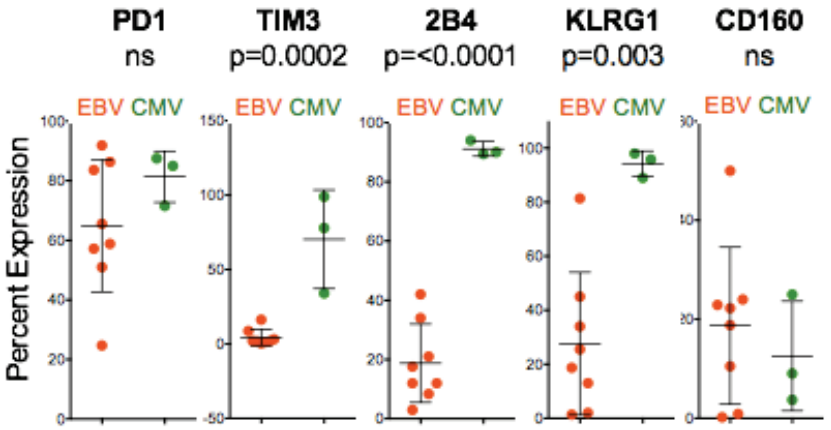


Figure 3. Differential expression of activation/exhaustion markers on donor EBV- and CMV-specific T-cells



In the proposed trial, we plan to first demonstrate safety of autologous, polyclonal T_N and T_{CM}-derived cells transduced with TCR_{C4}, and then ultimately broaden the components of the investigational product to include transduced $CD8^+$ T cells derived from an autologous EBV-specific population. The modified design will allow for comparison of persistence of EBV-specific T cells (which, as described above, commonly express T_{CM} characteristics, but also may get boosted *in vivo* by host EBV antigens) with both T_N- and T_{CM}-derived cells within individual patients, allowing for investigation into whether an EBV-specific TCR is responsible (necessary) and/or contributes to the observed T cell persistence in treated patients. Although it is likely that in some patients a subset of autologous, polyclonal T_{CM} cells selected for transduction will, in fact, be EBV-specific, the proportion is expected to be small. Monitoring of WT1/EBV bi-specific cells based on their ability to bind WT1- and EBV-specific tetramers will allow for determination of the proportion of TCR_{C4}-transduced cells derived from the EBV-specific subset.

3.H. Rationale for Lymphodepleting Chemotherapy Prior to T Cell Therapy

Transfer of T cells into lymphopenic hosts results in enhanced cell expansion of T cells with a memory phenotype and with enhanced effector function.[99,107] The favorable impact of lymphodepleting conditioning on survival and efficacy of transferred T cells has been observed in animal models,[108-111] donor lymphocyte infusions post-stem cell transplant,[112] and in numerous clinical trials with adoptive T cell transfer in melanoma and other cancers.[113-115] There are several proposed mechanisms by which lymphodepletion augments T cell expansion and function. These include removal of the potential competition with endogenous T cells for homeostatic cytokines such as IL-7 and IL-15, as evidenced by the fact that proliferation is reduced in a dose-dependent manner when “irrelevant” T cells are infused along with a T cell population of interest.[116] Lymphodepleting chemotherapy may also significantly decrease $CD4^+$ $CD25^+$ regulatory T cells, which have been shown to suppress *in vitro* function of tumor-reactive T cells.[117,118]

Our clinical experience with TCR_{C4}-transduced cells on FHCRC Protocol 2498 is limited to patients who have received T cells following post-transplant engraftment and count recovery, and has not included lymphodepleting chemotherapy, as there was concern about the adverse effects of chemotherapy on engraftment in the early post-transplant period. In the proposed trial, we plan to first demonstrate safety of autologous, polyclonal T_N and T_{CM}-derived cells transduced with TCR_{C4} in an initial cohort of leukemia patients, and in the subsequent cohort investigate the impact of lymphodepleting chemotherapy on safety, persistence and possibly efficacy. For the

proposed trial, a combination of cyclophosphamide and fludarabine will be employed, as has been demonstrated in several clinical trials, with proposed doses for our study in line with the current CD19 CAR T-cell trial **FHCRC protocol 2639,** as well as ongoing pediatric studies **(FHCRC/Seattle Children's PLAT-02 protocol)** .[112,114,119-121]

3.I. Safety Concerns

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

Low level WT1 expression can be detected in normal kidneys, testes, ovary, pleura, pericardium, the uterus and fallopian tubes, and $CD34^+$ hematopoietic progenitor cells [55]. 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 mutated to achieve an affinity ~200-500 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.[122]

The safety of murine T cells transduced to express the enhanced affinity TCRs in this study raised the question of why these TCRs were not detected in the normal repertoire. Therefore, we transduced these TCRs into HSC and examined thymic T cell development and maturation. We found that, unlike developing T cells 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 survive negative selection and emigrate from the thymus, but exhibit down-regulation of the TCR and/or CD8, resulting in peripheral T cells with lower avidity than T cells expressing the naturally isolated TCR. These results suggest that negative selection in the thymus is overprotective for WT1-reactive T cells; 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 T cell clone isolated from a naturally occurring peripheral T cell should be low.

3.I.2 Previous clinical experience: targeting WT1 with adoptive 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 damage to organs expressing physiologic levels of WT1, including no hematopoietic suppression or graft failure, renal failure, pleuritis, pericarditis, abdominal pain (from splenic capsule or ovaries) and/or testicular or ovarian pain.[77] 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 therapeutic potential 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 >70 HLA*0201$^+$ donors. Thus, the T cell expressing this TCR had undergone negative selection during thymic development.

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. Although determined largely by the affinity of the expressed TCR, expression of co-stimulatory and adhesion molecules and their ligands 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 T2 B-LCL cells (**Figure 4**, below), a transporter associated with antigen processing (TAP)-deficient B lymphoblastoid cell line, pulsed with decreasing concentrations of $WT1_{126\text{-}134}$ peptide. The TCR_{C4}-transduced CD8 T cells exhibited a functional avidity similar to the best WT1-specific CTL clones infused into patients in our previous study[77] and to the parental C4 clone from which the TCR was derived. Of note, a subset of the clones with similar high avidities persisted long-term (>100 days), without toxicity.

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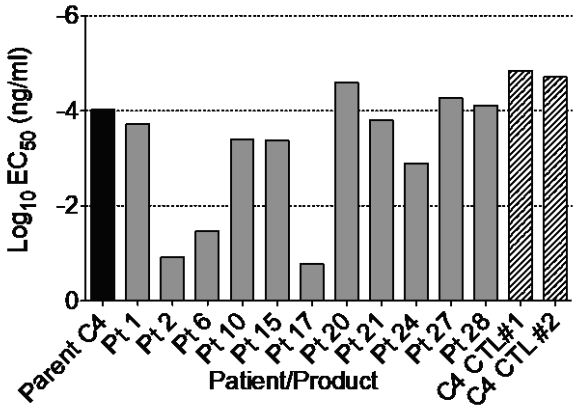

Figure 4. Avidity of products to WT1-pulsed T2 B-LCL

3.J. 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 either are at a high risk of disease recurrence or have already relapsed, in a phase I/II clinical trial (**protocol FHCRC #2498**) designed primarily to evaluate the safety and efficacy of intra-patient escalating doses. For the current trial, TCR_{C4} is being introduced into HLA-A*0201-restricted Epstein–Barr virus (EBV)- or cytomegalovirus (CMV)-specific donor cells. The use of virus-specific cells serves three purposes: 1) it 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) it removes the possibility of transducing T_N cells that contribute disproportionally to GVHD following HCT [123,124]; and 3) it ensures the transduced T cells will contain a large fraction of T cells derived from the T_{CM} cell pool, which have properties that enhance persistence after transfer.

3.J.1 FHCRC #2498 Study Schedule

Initially, this protocol planned for each patient to receive a total of 4 infusions following a dose-escalation schedule: 1 x 10^9 cells/m^2, 3.3 x 10^9 cells/m^2, 1 x 10^{10} cells/m^2, and 1 x 10^{10} cells/m^2 with this last dose followed by low-dose subcutaneous (s.c.) Interleukin-2 (IL-2) at 2.5 x 10^5 IU BID x 14 days, administered to enhance the survival of transferred T cells.[125] 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 3-6 patients (Arm 1/Stage 1) were planned 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 planned to receive infusions on the ‘Treatment Arm (Arm 2). The first 3-6 patients (Arm 2/Stage 1) were planned to receive the same infusion doses as Arm 1/Stage 1, but these were planned to be separated by a shorter interval to reach higher and potentially therapeutic doses in a more 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. The initial treatment plan is shown in **Figure 5**, below.

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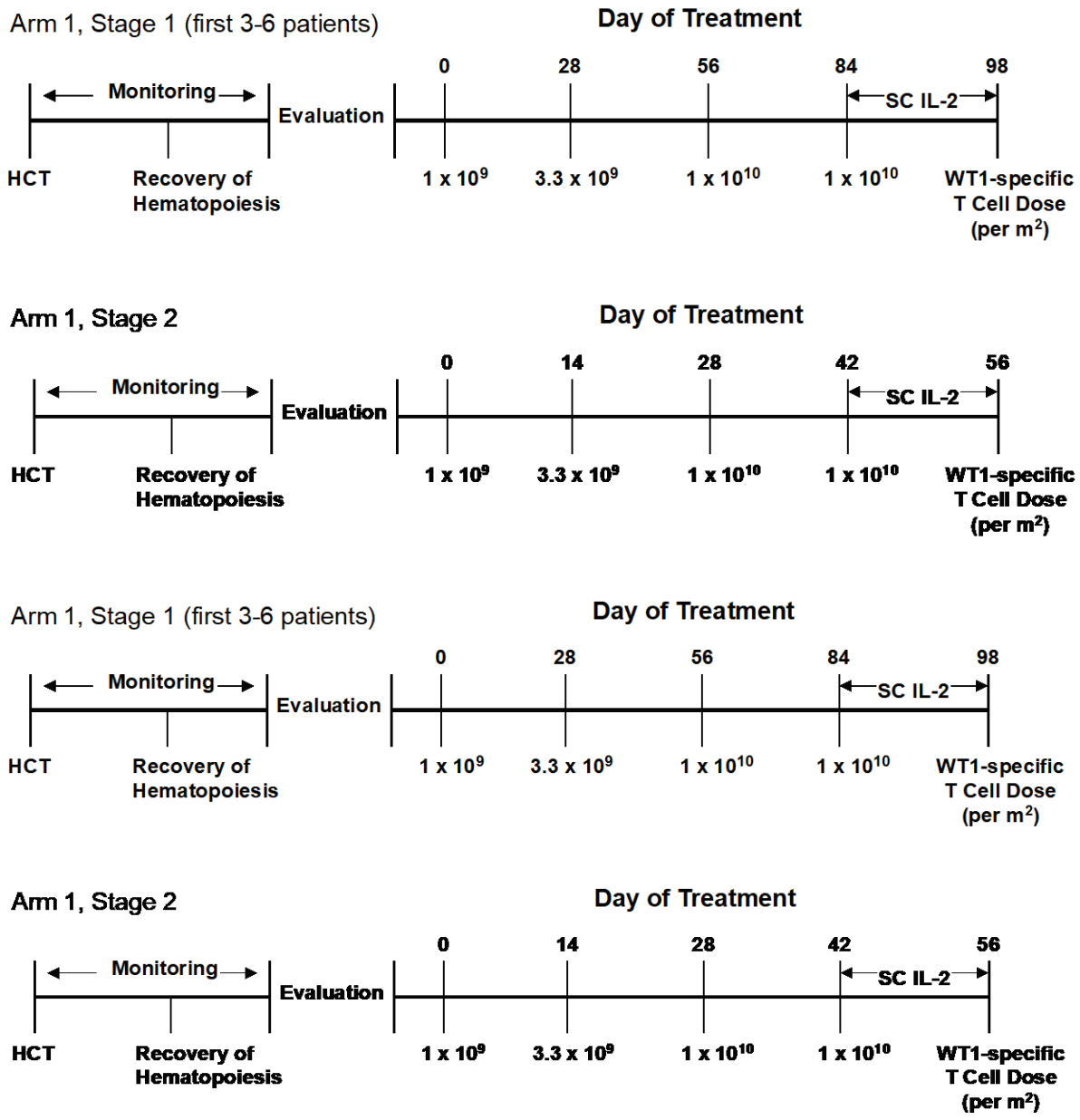
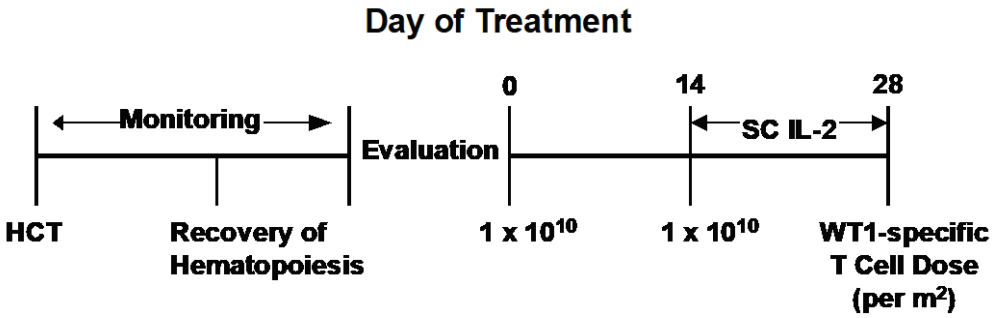
Figure 5. Initial treatment plan of protocol 2498, Arms 1 and 2



Because the dose-escalation schedule was well tolerated and no severe or unexpected toxicities were observed in the first 7 patients treated with up to 10^{10} TCR_{C4}-transduced cells/m^2, 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) have been receiving a first dose of 10^{10} cells/m^2 on day 0, and a second infusion of 10^{10} cells/m^2 at least 14 days after the first infusion, or later if the persistence of the cells from the initial infusion

is >3% of total CD8+ cells. The second infusion is followed by 14 days of s.c. low-dose IL-2 (**Figure 6**).

Figure 6. Revised treatment plan of protocol 2498, irrespective of assigned Arm



3.J.2. Patient characteristics on Protocol 2498

As of December 1, 2016, 25 patients with high-risk AML received a total of 48 doses of donor-derived virus-specific cells (**Tables 1A and 1B**). Thirteen patients received prophylactic treatment on Arm 1 and twelve patients were treated on Arm 2 due to detectable disease post-HCT. Overall, 33 of the 48 T-cell infusions represented the maximum target dose of 1×10^{10} WT1-specific CTL/m^2, and, of those, 11 infusions were followed by low-dose s.c. IL-2.

Table 1A. Patient characteristics on Protocol 2498, Arm 1

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.	Age/ Gender	Disease	EBV/ CMV	Arm	Comments
3	49 M	AML with complex cytogenetics, received HCT after 2nd CR	EBV	1	Poor T cell persistence, remains in remission 43 months after transplant
10	64 M	high-risk AML, received HCT in CR No evidence of disease after HCT.	EBV	1	Persistent T cells, in remission 30 months after HCT.
11	59 F	High risk AML, received HCT in CR No evidence of disease after HCT.	EBV	1	Persistent T cells, remains in remission 36 months after HCT.
12	55 F	HCT at CR1, high risk AML. No evidence of disease after HCT	EBV	1	Persistent T cells, remains in remission 33 months after transplant
13	59 F	high-risk AML, HCT in CR1, no evidence of disease after HCT	EBV	1	Persistent T cells, remains in remission 30 months after transplant
16	65 M	MDS-> AML, 5.5% blasts at HCT.	EBV	1	Poor T cell persistence, remains in remission 28 months after transplant
17	30 M	Extramedullary AML, MLL gene-rearrangement.	EBV	1	Poor T cell persistence, remains in remission 26 months after transplant

18	47 M	Relapsed AML in CR2	EBV	1	Persistent T cells, remains in remission 19 months after transplant
19	77 F	Relapsed AML in CR2	EBV	1	Persistent T cells, remains in remission 18 months after transplant
21	59 F	AML with complex cytogenetics, FLT3-ITD mutation.	EBV	1	Persistent T cells, remains in remission 13 months after transplant
22	30 M	AML with complex cytogenetics	EBV	1	Relapsed disease (extramedullary and cytogenetic relapse in marrow) at 8 month after transplant; currently in remission afte local and reinduction systemic therapy
24	57 M	AML in CR2; MRD at time of SCT	EBV	1	Persistent T cells, remains in remission 10 months after transplant
25	67 F	MDS -> AML, poor-risk cytogenetic	EBV	1	Persistent T cells, remains in remission 4.5 months after transplant

Table 1B. Patient characteristics on Protocol 2498, Arm 2

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.	Age/ Gender	Disease	EBV/ CMV	Arm	Comments
1	56 M	AML, relapse with para-spinal chloroma 5 years after 1st myeloablative HC	EBV	2	Disease progression. Removed from study before 4th infusion
2	51 F	AML, 2nd HCT for relapse 9 years after 1st myeloablative HCT. Entered HCT with 16% leukemia blasts and blasts were again detectable after 2nd HCT	CMV	2	Normalization of counts and decreased abnormal blast population to 0.006% after T cell infusions.
4	25 M	AML, relapse with medullary and extra medullary disease after 2nd HCT	EBV	2	Patient remained in CR for 1 year after infusion, then relapsed with extramedullary disease when WT1+ CD8+ cells measured at >3% of total CD8 cells. Disease progressed despite 2nd infusion.
5	49 M	AML, disease progression after HCT (70% PB blasts at T cell infusion)	EBV	2	Disease progression. Off study at 6 days, and expired 12 days after 1st infusion.
6	20 F	AML, second HCT for relapse 2 years after 1st myeloablative HCT	CMV	2	Poor T cell persistence (0.04% at day 173), in CR 24 months after HCT.
7	33 F	AML, MRD after myeloablative HCT	EBV	2	Progressive disease. Received Azacitidine after 1 T cell infusion.
8	63 F	AML, received HCT in CR2. MRD early after transplant	EBV	2	Persistent T cells, disease progressed after infusion, and now receiving systemic therapy.
9	67 F	AML, received HCT in CR2. Relapse early after transplant	CMV	2	Poor T cell persistence, progressive disease.
14	17 M	AML, 2nd HCT for relapse 5 years after first HCT.	EBV	2	Disease progression despite persistent T cells; received azacitidine.
15	69 F	MDS -> AML, second HCT for relapse 1 year after 1st HCT	CMV	2	Disease progression despite persistent T cells.
20	5 F	Relapsed AML after transplant; active disease (chloromas); MLL gene-rearrangement.	EBV	2	Disease progression despite persistent T cells; receiving re-induction chemotherapy
23	75 M	Relapsed AML after transplant	EBV	2	CR at time of T cell infusion; continued complete remission

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3.J.3. Persistence of TCR_{C4}-transduced cells on Protocol 2498

Patients who received cells generated from EBV-specific cells generally demonstrated a high-level of persistence. Of the 14 patients who received transduced EBV-specific cells and could be followed beyond 4 weeks after their latest infusion, 10 had persistent frequencies >3% (range 3-60% of CD8 T cells) for an average duration of 44 weeks (range 3-85 weeks) (**Figures 7A and 7B**, below) representing an average maximum of 215 cells/microliter (range 10-989 cells/microliter). The remaining 4 patients who received transduced EBV-specific cells exhibited frequencies <3% within 1-14 days. Patient 4, who received the lowest dose (1×10^9), had cells detectable in the blood at frequencies of 4% to 8% of total $CD8^+$ T cells until 14 months after infusion, with a decrease to <3% by 16 months after infusion. Until adequate long-term safety is formally demonstrated, patients cannot receive additional T cell infusions if their WT1-specific $CD8^+$ T cell frequency from the previous dose is ≥3% of total $CD8^+$ T cells. Therefore, at this time, Patients 8, 10, 11, 12, 18, 19 and 20 have not received a second infusion. Of the 4 pts (Pts 2, 6, 9 and 15) who received CMV-transduced cells, Pt 2 demonstrated persistence of the TCR_{C4}-transduced cells for 49 weeks after the final infusion at frequencies well below 1%. Pts 6 and 9 had no persistence beyond 4 and 2 weeks, respectively, after their last infusion. Pt 15, however, had persistence of the TCR_{C4}-transduced cells above 7%, with the last follow-up 68 days after infusion.

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Figure 7A. Observed frequencies of infused WT1-specific T cells (% CD8+ cells) in Arm 1 patients.

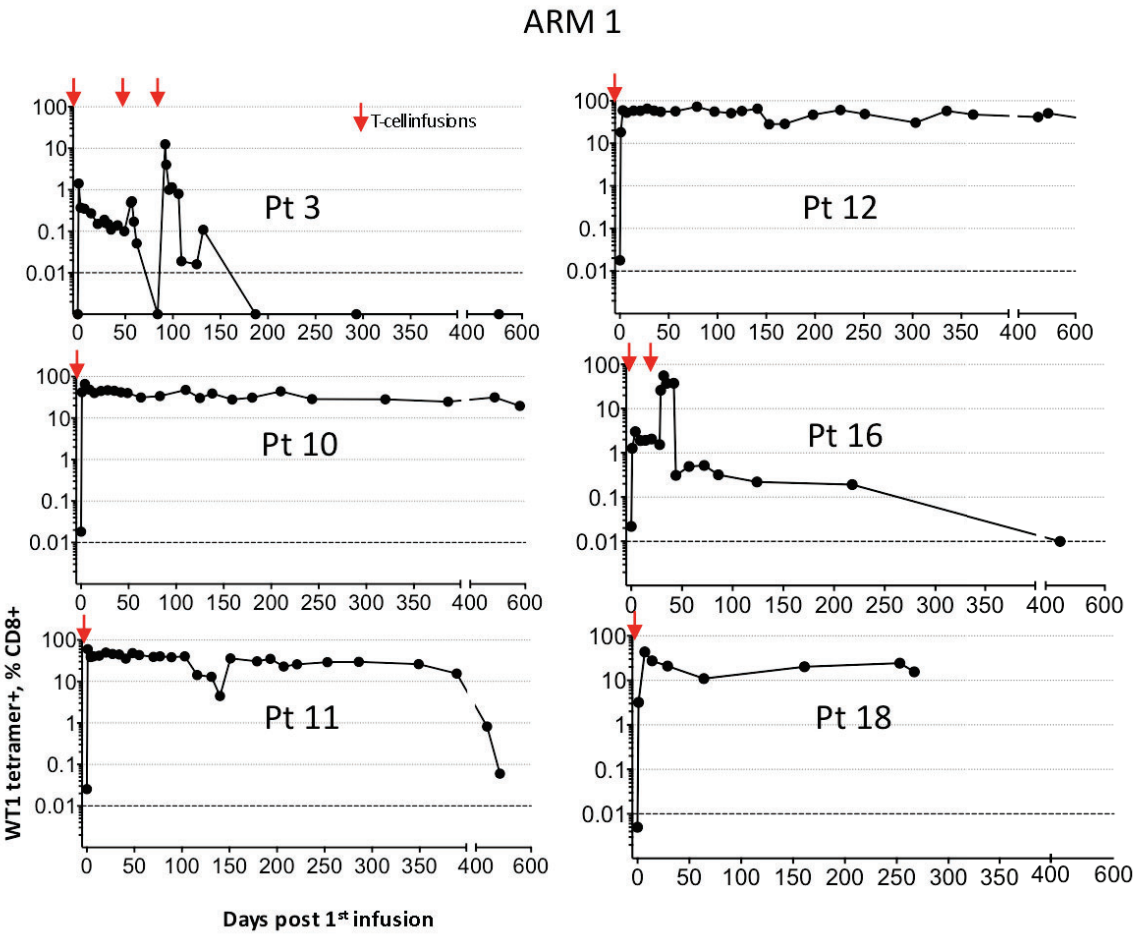

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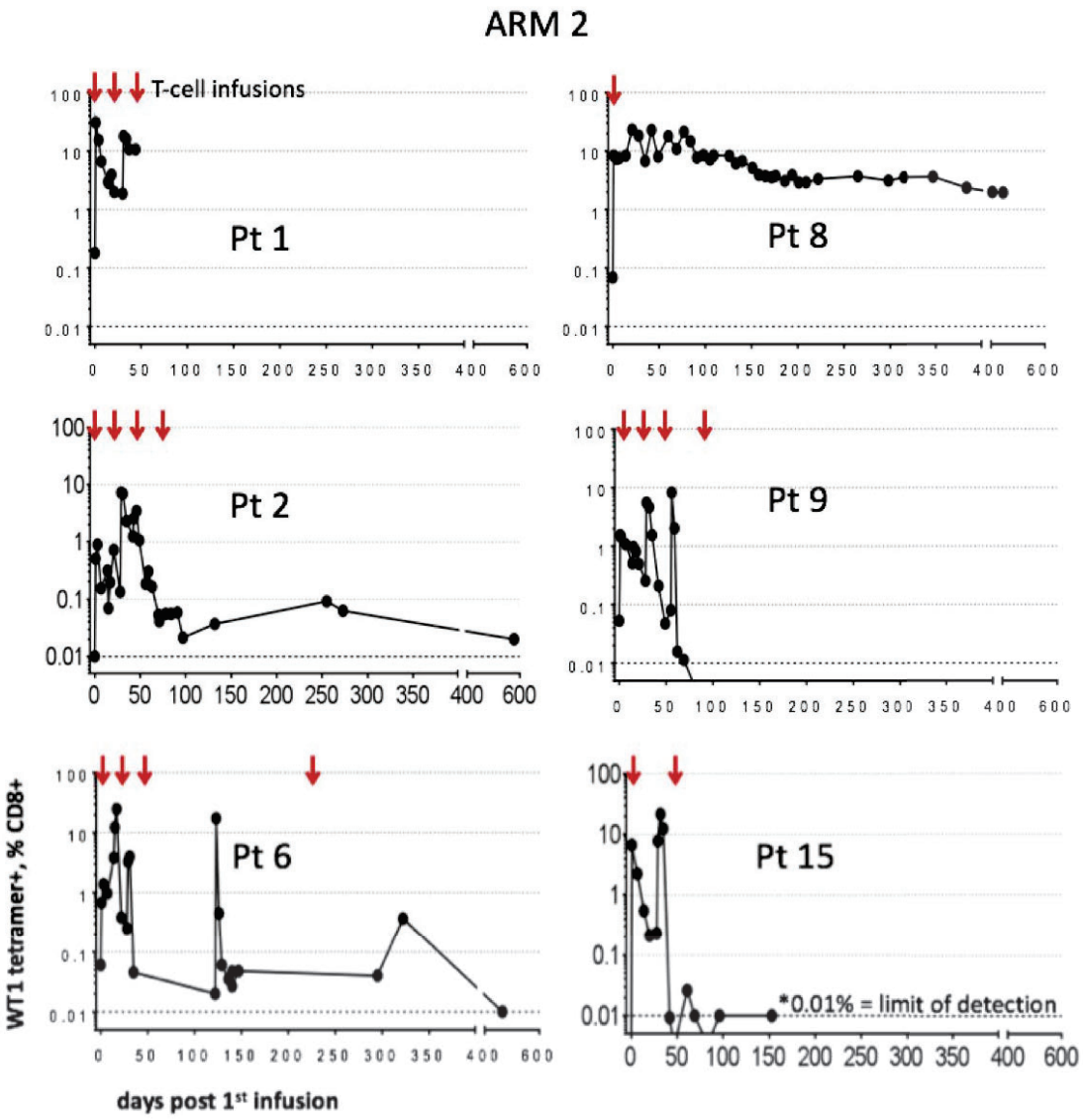
Figure 7B. Observed frequencies of infused WT1-specific T cells (% CD8+ cells) in Arm 2 patients.



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3.J.4 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	3		
	Fatigue	1	1		
	Tachypnea	4			
	Hypotension	5	2	4	
	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	2	
	AST increased	8		2	
	Alkaline phosphatase increased	4		1	
	Creatinine increased	3			
	Hypoalbuminemia	4			
	Hypocalcemia	7			
	Hypomagnesemia	1			
	Hyponatrema	5			
	Hypokalemia	3			
Miscellaneous	Dry eyes		2		
	Decreased respiratory rate	4			
	Maculopapular Rash			1	

Expected transient symptoms of **cytokine release syndrome** (CRS) were observed, as this syndrome is 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, 9 patients experienced fevers (≥ 38.3°C) with or without accompanying chills, including 2 patients with fever > 40.0°C following the infusion. Blood cultures were negative for bacterial or fungal growth in all cases. Many patients also experienced ≤ 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 two patients 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 in the outpatient General Clinical Research Center (GCRC) or on the general hospital ward without ICU support.

Importantly, we have not observed any on-target/off-tissue toxicities in any of the 20 patients who received WT1-specific T cell infusions. Specifically, there has not been any observed graft failure, renal failure, nephrotic-range proteinuria, pleuritis, pericarditis, or testicular or ovarian pain in any of the study cohort. 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.[15,126] The temporary drop in total lymphocyte counts returned to pre-infusion levels within 7 to 11 days in all patients. Three cases of grade 3 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, [127] and to date all patients treated on this study started the T cell infusion with existing 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 8**, below).
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 (e.g., electrolyte abnormalities are common side effects of calcineurin inhibitors and antifungals; elevated liver enzymes are a common side effect of antifungals), poor nutritional status (hypoalbuminemia), and secondary infections.

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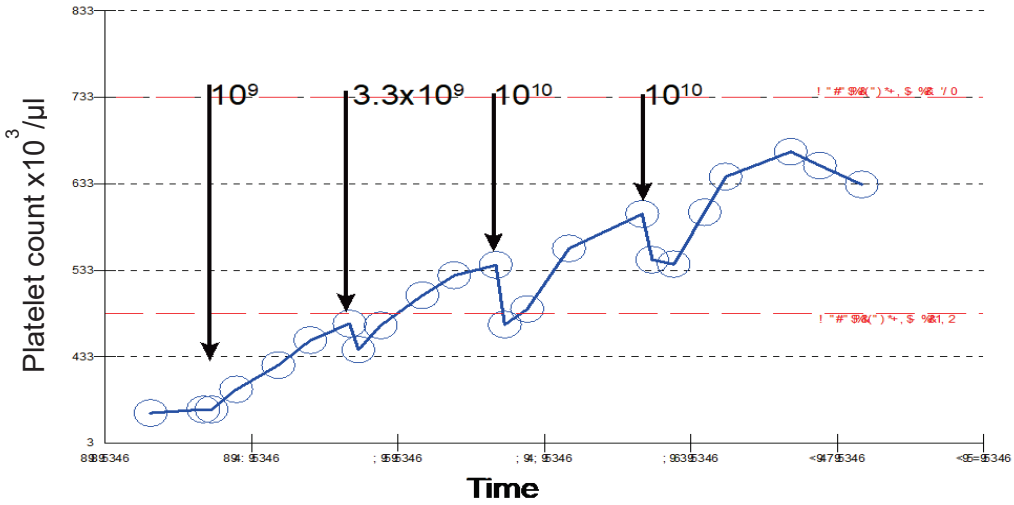
Figure 8. Kinetics of platelet counts after infusions (Pt. 2)



One patient (Pt 6) without any prior GHVD was diagnosed at 10 months after transplant, which was 4 months after her final WT1-specific T cell infusion, with grade III late acute GVHD affecting liver (stage III), GI tract and skin, as well as chronic GVHD involving oral mucosa and eyes. Symptoms and transaminitis responded favorably to steroids. A second patient (Pt 13) was diagnosed 13 months after transplant, which was 10 months after her final WT1-specific T cell infusion, with grade III late acute GVHD affective liver (stage III). These are the only cases of grade III/IV GVHD observed in our study population, and notably WT1-specific T cells were near undetectable (0.04% of CD8+ cells) or undetectable, respectively, at the time of diagnosis making any association with the WT1-specific T cells unlikely.

One patient (Pt 16), with a prior history of acute GI GVHD, was diagnosed with chronic GVHD affecting liver (stage I, transaminitis only) and oral mucosa at 7 months after HCT, which was 3.5 months after WT1-specific T cell infusion. As above, WT1-specific T cells were undetectable at the time of the transaminitis, making any association with WT1- T cells unlikely. A patient (Pt 11) with a history of acute GVHD involving the skin and GI tract had a GVHD flare in the setting of tapering tacrolimus at 3 months following WT1-specific T cell infusion. Two patients (Pts 2 and 10) developed chronic GVHD after treatment with WT1-specific T cells, one of whom had a prior history of acute GVHD.

One patient (Pt 8) was diagnosed with acute GVHD affecting the skin and GI tract at 2.5 months after HCT, which was 7 days after receiving her first infusion of WT1-specific T cells. Symptoms

were mild and responded to prednisone. This patient also had a prior history of cryptogenic organizing pneumonia (COP) occurring before transplant, and was diagnosed with a flare of COP at 6.5 months after HCT, which was 4 months after infusion of WT1-specific T cells. Initially responsive to steroids, she had a repeat flare of COP 4 months later while tapering prednisone, and required continued steroid therapy.

3.J.5 Efficacy observed on protocol #2498

Arm 1: Thirteen patients with high-risk leukemia entering HCT (**Table 3**, below), and who had no detectable disease after HCT, received WT1-specific cells within a median of 104 days post-HCT. With a maximum follow-up of 849 days and a median of 407 days after HCT, all patients are alive in CR and have not relapsed to date (**Figure 9**). For comparison, patients undergoing HCT with high-risk disease have an ~30% chance of relapse at 1 year.[16-20] This suggests the infusion of WT1-specific cells in the post-HCT setting may prevent leukemia recurrence.

Arm 2: Eleven patients with high-risk leukemia entering HCT with detectable disease after HCT had a higher cumulative risk based on adverse factors compared to pts treated on Arm 1 (**Table 4**, below). Patients received T cell infusions at a median of 77 days after HCT. Although 2 of 11 patients remain free of disease, with a median follow-up of 206 days after the first T cell infusion, 44% are still alive (**Figure 10**, below). For comparison, patients who relapse within 6 months after HCT have <10% chances of being alive at 1 year.[128] This suggests WT1-specific T-cells may confer a survival advantage despite the absence of complete clearance of relapsed AML post HCT.

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Table 3. Cumulative risk factors for patients on Arm 1

Pt.	>CR1	Cytogenetics	refractory (>1cycle to achieve CR)	Disease at HCT	MDS->AML or secondary AML	Chloroma
3	X	X				
10		X	X			
11		X	X			
12		X (FLT3+)		MRD (cyto)		
13		X	X			
16				5.5% blasts	X	
17		X (MLL)				X
18	X					
19	X					
21		X (FLT3+)				
22			X			
24		X			X	
25	X			X		

All patients have received EBV/WT1 bi-specific cells.

Figure 9: Progression-Free Survival of Arm 1 since HCT

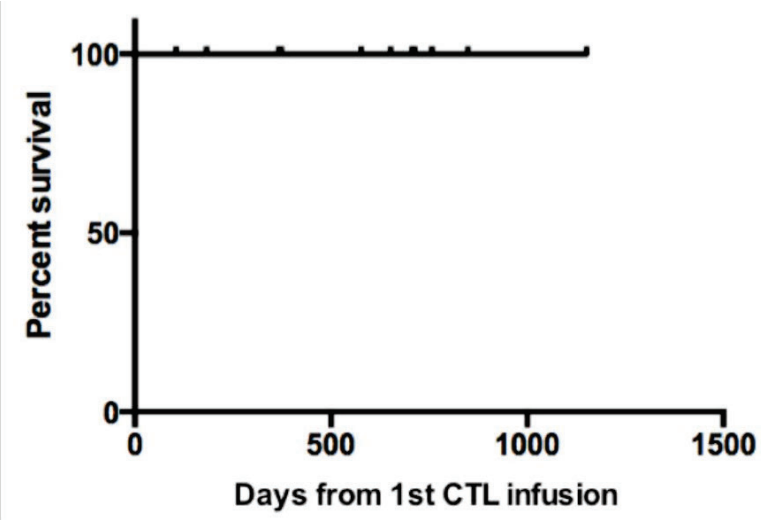

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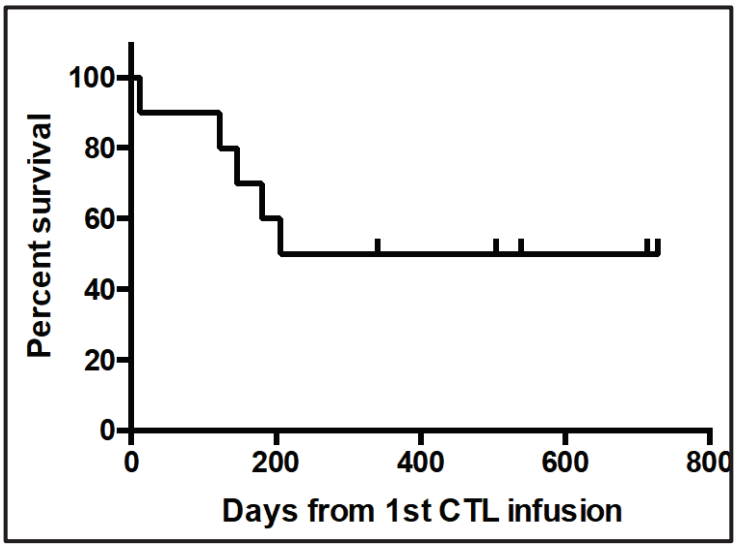
Table 4. Cumulative risk factors for patients on Arm 2

Pt.	>CR1	Cyto-genetics	Refractory (>1cycle to achieve CR)	Disease at HCT	Secondary AML	Chloroma	2nd Transplant
1	X	X	X		X		
2	X	X	X	16% blasts			X
4	X	X	X	0.02% blasts		X	X
5	X	X	X	42% blasts	X		
6	X	X	X				X
7	X	X	X	3.8% by cyto			
8	X	X	X	0.01% blasts	X		
9	X	X		no counts			
14	X	X	X	0.02% blasts	X		X
15	X	X	X		X		
20	X	X				X	
23		X	X	X			

Figure 10: Overall survival of Arm 2 since 1st CTL infusion



3.J.6 Protocol #2498: Conclusions to date

To date, WT1-specific T cells were detectable for a cumulative total of 471 weeks in 25 treated patients, and at 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 T cells transduced with the WT1-specific TCR_{C4} did not appear to injure normal tissues expressing 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.

In the proposed trial, patients will receive a first dose of 1 x 10^9 cells/m^2 and then potentially a second dose of 1 x 10^{10} cells/m^2 followed by low-dose s.c. IL-2. Although we cannot formally exclude the possibility of inducing tissue toxicity in the proposed trial, there is no evidence for such toxicity in our ongoing trial with the same TCR or in our previous trial with WT1-specific CD8 CTL clones.

3.K. Rationale 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. However producing such a high cell number is very expensive and takes several weeks. Therefore, in this study we would like to evaluate the potential persistence and function of smaller cell doses. Thus, we will start with infusion at a dose of 1 × 10^9 cells/m^2 with intra-patient dose escalation up to 1 × 10^{10} cells/m^2. In order to evaluate persistence, the second dose will be held if the transferred cells persist at a level of > 5% of the total $CD8^+$ cells and at an absolute number of >30 cells/µl.

3.L. 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 [125]. It was apparent, however, that the *in vivo* frequency of transferred CTL began to drop before the end of the 14-day course, suggesting continuing IL-2 administration beyond this time may be nonproductive or may even be counter-productive, as it could 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 associated with IL-2 activity, including administration of IL-2 alone, of adoptively transferred lymphokine-activated killer (LAK) cells generated by culture in high dose IL-2, and of tumor infiltrating lymphocytes (TIL) expanded by culture with IL-2.[129-131]

These studies included administration of high doses (up to 7.2 x 10^5 units/kg 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 IL-2 receptors, and can therefore induce non-specific widespread activation of NK cells and T cells that can likely mediate most/all of the observed toxicities via cytokine release and/or 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 immuno-modulatory effects of administering lower doses of IL-2. Doses of 1.25 x 10^5 to 5 x 10^5 U/m^2/day administered s.c. are well tolerated for up to 84 days.[132] In cancer patients, there is little evidence for anti-tumor activity from administration of low dose IL-2 alone. However, studies from our group in 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.[133]

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/survival with minimal toxicity.[125] Patients previously treated with donor-derived $CD8^+$ T cell clones after HCT [77] 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.M. Justification for using HTTCS for determining cellular subpopulations

Next-generation sequencing techniques, so-called high throughput TCRβ chain sequencing (HTTCS) as demonstrated by Robins et al, may be used to accurately sequence the sampled TCR repertoire.[103-105] In the proposed study, transduced T cells will each contain a unique, endogenous T cell receptor that will be quantifiable by HTTCS in the generated immunotherapy product. In essence, this “bar coding” of the varied T cell clonotypes allows for post-infusion assessment of clonal dynamics within a single patient. This ability to track the individual fates of cells comprising the infused polyclonal cells with HTTCS has been demonstrated following infusion of MART1-specific T cells in a melanoma population. Indeed, the sum of MART1-specific TCR clonotypes was shown to correlate well with the overall proportion of MART1-specific T cells as assessed by multimer staining.[134] Furthermore, tracking individual TCR sequences made it possible to identify the emergence in a patient of a single immuno-dominant clonotype within 3 months after infusion of the cells (**Figure 11**). In our proposed study, HTTCS will be performed initially on the generated T_N and T_{CM} subsets, allowing for accurate measurement of T_N-derived and T_{CM}-derived cells over time. In a preliminary analysis using a 1:1 mixture of WT1-specific T_N and T_{CM} cells in 3 lung cancer patients, the endogenous TCR composition of the T_N and T_{CM} products was assessed. Unique TCR sequences (comprising at least >0.01% of each potential infusion product) accounted for 89-94% of total T_N sequence reads and 88-97% of total T_{CM} sequence reads (**Figure 12**). Thus, HTTCS should allow accurate discrimination between the daughter cell derivatives of these two populations in the vast majority of cases, with the only exceptions being if immunodominant clonotypes evolve *in vivo* that are derived from the small % of overlapping sequences that need to be excluded from the analysis.

Figure 11. Frequency and dynamics of MART1-specific TCR clonotypes in a melanoma patient, as determined by HTTCS.

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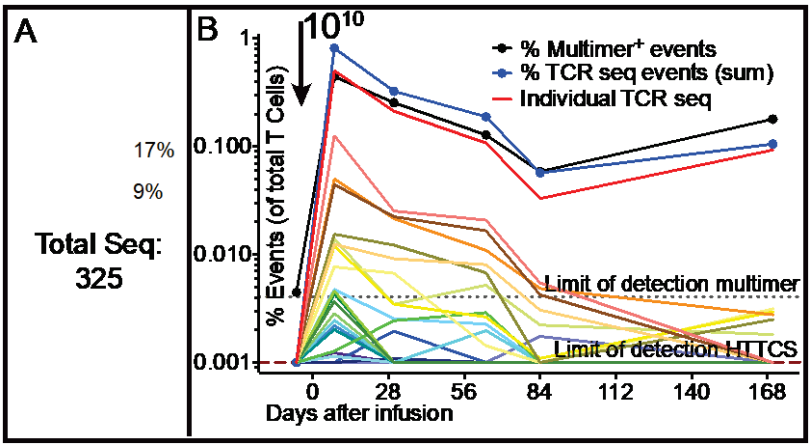
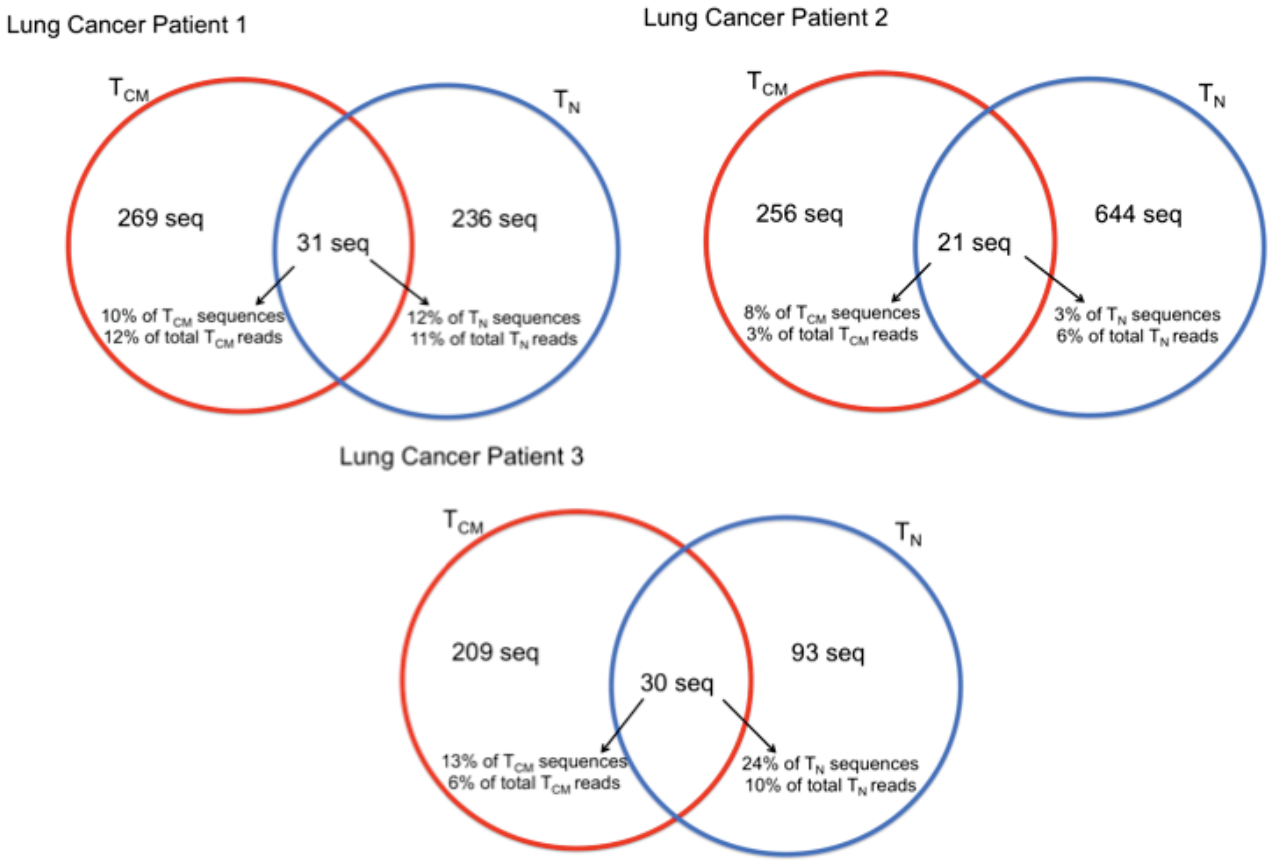


A) Relative frequencies of 325 clonotypes in the infusion product at baseline. **B)** Dynamic *in vivo* frequencies of the infused TCR sequences. Sum of clonotypes (blue solid circles) was concordant with the proportion of MART1-specific T cells as measured by multimer staining (black solid circles). Despite 325 clonotypes being detected in the starting donor population, nearly half of the infusate was derived from 3 clonotypes – tracking of individual TCR seqs (colored lines) revealed that these 3 represented >90% of cells detected 7 days after infusion, and 1 immunodominant clonotype was persistent 168 days after infusion (red line).

Figure 12. Unique and overlapping TCR sequences in TN and TCM subsets after generation of WT1-specific T cells for use in 3 lung cancer patients



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3.N. Rationale for Enrollment of Pediatric Patients

In children and adolescents, AML accounts for approximately 20% of acute leukemias. Although outcomes are better in pediatric patients overall, with 60-65% of patients with intermediate risk achieving long-term survival,[33] patients with selected high-risk features, including defined high-risk genomic aberrations or persistent disease, continue to demonstrate poor survival with less than 50% OS at 5 years.[34,135,136] The indication for HCT in non-high risk pediatric AML remains controversial, although retrospective analysis of Children's Oncology Group data suggests a survival benefit with HCT in pediatric patients in first CR classified as having intermediate risk disease.[137] It remains unknown whether similar long-term outcomes might be observed with deferment of HCT until relapse. As with adults, the prognostic impact of MRD at the end of induction has been demonstrated in pediatric patients, with ~30% DFS in MRD-positive patients compared to 65% in those without detectable disease.[136] At higher levels of MRD ≥0.5% at the end of induction, only 15% of patients are alive at 3 years without disease.[74] Thus, as per the practice of the Seattle Children's pediatric oncology group, HCT is generally indicated in pediatric patients with MRD at end of induction.[58,138-141] Therefore, we will consider pediatric patients with MRD or incomplete count recovery, but who cannot undergo HCT, as eligible to receive the study intervention. Patients must be ≥15kg, as patients with lower weight would be incapable of providing high volume and frequent blood samples for monitoring and analysis. While the number of pediatric patients with these high-risk features who cannot proceed to transplant is admittedly expected to be small, the very poor prognosis justifies consideration of the study intervention in this group.

3.O. Rationale for modification of eligibility criteria to include patients with "high-risk" AML (who do not undergo transplantation), irrespective of MRD status or presence of post-induction incomplete count recovery

3.O.1. High-risk cytogenetics or molecular classification

As of July 2018, a modification in the inclusion criteria has been made to allow for enrollment of a larger population of patients with "high-risk" AML who do not undergo allogeneic transplantation, regardless of presence of MRD or poor count recovery. The clinical practice of incorporating cytogenetic and molecular data (e.g. complex karyotype, or FLT3-ITD mutation) to risk-stratify patients with AML is long-standing and well-validated. Acknowledging that cytogenetic and molecular risk classifications change over time, the 2017 European LeukemiaNet guidelines are

hereby being considered as a recent and reliable reference for current reference; these widely-accepted guidelines are now incorporated into our determination for high-risk disease features that should be considered for eligibility for our protocol.[142] We propose to include, in our trial, these other AML patients who, despite lack of MRD or CRi, remain at a very high risk for disease relapse without transplant.

Because of the risks of morbidity and mortality associated with SCT, there remains some controversy as to who should or should not undergo SCT. That being said, it is generally accepted that patients with “adverse risk” AML disease should be considered for SCT due to its curative potential, and because of the overall poor outcomes in this population.[142] In support of this practice, a 2009 meta-analysis of many prospective trials showed that relapse is reduced and long-term survival is improved with SCT compared to chemotherapy alone in patients with adverse risk AML.[5] The median time to relapse in such “adverse risk” patients who do not undergo SCT is reported to be approximately 12 months, which is actually quite similar to the rate of relapse in the original study population for this study (AML patients with MRD or CRi). Notably, the rate of relapse in patients with adverse cytogenetic or molecular features has been reported at 70-90% by 2 years in multiple publications.[3,44,143,144] Additionally, patients with an initial disease remission but who develop a subsequent relapse have a very poor prognosis, with an estimated survival of only 5-10% at 2 years, with death mainly due to disease relapse.[145,146] Therefore, such “high-risk” patients who do not undergo SCT, regardless of MRD or count recovery, are considered to have similar risks for disease relapse to the initially proposed study population, and their inclusion in the study should improve accrual, while maintaining the ability to observe (within 1-2 years) any impact of WT1-specific T cells in reducing AML relapse as compared to the proposed contemporaneous “observation” cohort that does not undergo SCT and that does not receive the study intervention.

3.O.2. Cytogenetic and molecular minimal residual disease (MRD) as a “high-risk” criterion

In addition to the established prognostic significance of cytogenetic abnormalities at the time of diagnosis, it has become increasingly apparent that the persistence of detectable leukemic-associated cytogenetic abnormalities (so-called “cytogenetic MRD”) is also negatively associated with survival and remission outcomes. In one of the earliest retrospective studies to explore the impact of cytogenetic MRD, Marcucci et al found a cumulative incidence of relapse of 100% by 3 years, and an associated

3-year disease-free survival of zero, in patients with an abnormal karyotype at the time of morphologic remission.[147] Importantly, high rates of relapse were observed across all cytogenetic risk groups. Similarly dismal outcomes were observed in a subsequent, larger retrospective analysis by Chen et al, showing that while allogeneic transplant was able to abrogate to some degree the adverse effect of cytogenetic MRD on relapse-free and overall survival, those patients with cytogenetic MRD (as defined by a persistently abnormal karyotype) and who did not undergo allogeneic transplant had a relapse-free survival of around 10% at 2 years.[148] Because of the markedly increased sensitivity of fluorescence in-situ hybridization (FISH) techniques in detecting persistent cytogenetic abnormalities, the possibility is raised that MRD detected by FISH alone may detect much lower levels of residual disease that may not be as strongly associated with relapse; indeed, the limited published literature, largely involving small numbers of patients, has found mixed results on this matter.[149-153] Thus, MRD by FISH alone will not be considered as a “high-risk” criterion in the present study.

Similarly, the landscape of molecular MRD is too complicated as to warrant prophylactic WT1-specific T cell immunotherapy in all patients with any type of “molecular MRD”. Indeed, the ELN MRD Working Party recommends against the use of Fms-like tyrosine kinase internal tandem duplication (FLT3-ITD), FLT3-TKD, NRAS, KRAS, IDH1, IDH2, and MLL-PTD as single markers of MRD because they are all prone to frequent losses or gains, or may otherwise be detected in healthy individuals at low levels.[154] Furthermore, mutations in TET2, DNMT3A and ASXL1 are still detectable during remissions of > 50% of patients, and are not clearly associated with relapse.[155]

However, there are clearly situations in which molecular MRD has been shown to be a strong risk factor for disease relapse, some of which are extremely relevant in a “non-transplant” AML population, including those patients with disease that is historically classified as “favorable” risk (e.g. core-binding factor AML or patients with normal karyotype and mutations in *NPM1*) and, thus, who tend not to be referred for allogeneic transplantation. Core-binding factor AML is defined by the demonstration of the specific gene rearrangements *CBFB/MYH11* and *AML1/ETO* (the latter also known as *RUNX1/RUNX1T1*), and of note these specific gene rearrangements correlate with inv(16) and t(8;21) cytogenetics, respectively. Recent analyses show that a subset of this “favorable” risk population is, nevertheless, at significant risk for relapse if molecular MRD is detected after therapy.

Several authors have shown that persistence of a relative or absolute copy number of abnormal transcripts (as compared to a control gene such as ABL) are highly correlated with relapse.[5,156-158] As an example, Yin et al demonstrated a 90% relapse risk in patients with persistently detectable inv(16) at greater than 10 absolute copies (compared to 10^5 copies of control gene ABL) in the marrow following at least 4 cycles of consolidation.[158] Of note, because the normalized copy number (NCN) method of reporting within the SCCA system is based on a 1:1 ratio of CB:ABL as opposed to a 1 *CBFB/MYH11* : 10^5 *ABL* ratio, as was reported in the study by Yin et al, this would correlate with a markedly increased risk in any patient with CBFB > 0.001 on the SCCA-specific NCN scale at any time after 4 cycles of consolidation. Similarly in that same study by Yin et al, a 100% risk of relapse without transplantation was observed in patients with > 500 copies of *RUNX1/RUNX1T1* (correlating with a value of 0.050 NCN in the SCCA system) at any time after 4 cycles of consolidation.

The log reduction in core-binding factor transcripts is also increasingly used in clinical practice to determine risk of relapse. Stentoft et al found that the log reduction in *CBFB/MYH11* and *AML1/ETO* at the time of post-induction disease restaging was also strongly predictive of relapse risk.[159] Using a threshold of ≥ 2 log reduction versus < 2 log reduction *in either blood or marrow* as a risk stratification, the observed rate of relapse was essentially 100% by 2 years in patients (who did not undergo a stem cell transplant) if a < 2 log reduction in the abnormal transcript was achieved after induction chemotherapy.

The persistence of detectable mutated NPM1 transcripts also appears to correlate with risk of relapse, and monitoring of NPM1 in the marrow or blood is recommended by the European LeukemiaNet guidelines. However, since the impact of NPM1 MRD positivity impacts survival and rate of relapse significantly less than those with MRD based on detection of core-binding factor transcripts or other groups outlined in the study (with NPM1 MRD, the 4-year cumulative incidence of relapse is more in the range of 50%), this group will not be considered as an eligibility criterion.[160-162]

Thus, we propose here that the following additional MRD categories be considered as “high-risk” for leukemic relapse in a non-transplant population and an acceptable eligibility criterion for our study: 1) cytogenetic MRD, as defined by a disease-specific abnormal karyotype at any point during a complete (morphologic) remission; 2) a normalized copy number (NCN) of > 0.001 for

CBFB/MYH11 or a normalized copy number (NCN) of > 0.050 for *AML1/ETO (RUNX1/RUNX1T1) after at least 4 cycles of consolidation chemotherapy;* or 3) a < 2 log reduction in either *CBFB/MYH11* or *AML1/ETO (RUNX1/RUNX1T1)* in the bone marrow at the time of post-induction disease restaging (immediately after 1-2 cycles of induction therapy).

STUDY OBJECTIVES

4.A. Primary Objectives

1. Determine the safety / potential toxicities associated with treating high-risk AML patients with autologous $CD8^+$ T cells (polyclonal T_N and T_{CM} cells; T_{EBV} that have been genetically-modified to express a high affinity WT1-specific TCR (TCR_{C4}).
2. Determine the feasibility of reproducibly treating high-risk AML patients with autologous $CD8^+$ T cells (polyclonal T_N and T_{CM} cells; T_{EBV} cells) that have been genetically-modified to express a high affinity WT1-specific TCR (TCR_{C4}).
3. Determine and compare the *in vivo* persistence in blood and at the primary tumor site (e.g. bone marrow, chloroma) of transferred autologous $CD8^+$ T cells (polyclonal T_N and T_{CM} cells; T_{EBV} cells) that have been genetically-modified to express a high affinity WT1-specific TCR (TCR_{C4}).

4.B. Exploratory Objectives

1. Determine whether adoptively transferred autologous TCR_{C4}-transduced $CD8^+$ cells have antitumor activity in patients with acute myeloid leukemia.

    1a. In patients with measurable MRD at the time of infusion of TCR_{C4}-transduced $CD8^+$ cells, changes in leukemic tumor burden will be measured by morphology, flow cytometry, cytogenetics/FISH and/or molecular testing at baseline and after infusion of T cells (see **Appendix C** for response criteria).

    1b. In all patients (those with or without measurable tumor burden prior to T cell transfer, including patients who convert to MRD-negative status during consolidation), the probability of relapse, disease-free survival and overall survival of patients receiving TCR_{C4}-transduced $CD8^+$ cells will be compared with patients in the observation arm.

2. Determine and compare the migration to the primary tumor site of subsets of the adoptively transferred autologous TCR_{C4}-transduced $CD8^+$ T cells (polyclonal T_N and T_{CM} cells; T_{EBV} cells).
3. Determine and compare the *in vivo* functional capacity of transferred polyclonal autologous TCR_{C4}-transduced $CD8^+$ T_{CM} ,T_N cells and T_{EBV} $CD8^+$ cells.

STUDY ENDPOINTS

5.A. Primary Endpoints

1. Evidence and nature of toxicities according to NCI CTCAE Version 4.0 (see **Section 15.E**)
2. Feasibility of generating TCR-transduced T_N and T_{CM} subsets for adoptive immunotherapy in a high-risk AML population (see **Section 15.F**).
3. Comparison of the relative frequencies and duration of persistence of adoptively transferred TCR_{C4}-transduced $CD8^+$ polyclonal T_{CM} and T_N cells, and of TCR_{C4}-transduced T_{EBV} $CD8^+$ cells in blood and at the primary tumor site(s) (see **Section 15.G**).

5.B. Exploratory Endpoints

1. Patients with measurable MRD at the time of infusion of TCR_{C4}-transduced $CD8^+$ cells: decrease in blast counts in blood or marrow (by morphology and flow cytometry), and/or decrease in disease burden as detected by cytogenetics/FISH or molecular testing (see **Appendix C** for response criteria).
2. Patients with or without detectable MRD: comparison of probability of relapse, disease-free survival (DFS) and overall survival (OS) to patients in the observation arm (see **Section 15.H**).
3. Relative frequencies at primary tumor sites of TCR_{C4}-transduced $CD8^+$ polyclonal T_{CM} and T_N cells and EBV-specific (T_{EBV}) cells compared to peripheral blood (see **Section 15.I**).

4. Relative maintenance of functional capacity and potential acquisition of phenotypic characteristics associated with T cell exhaustion in transferred $WT1^+$ cell populations T_N compared to T_{CM} compared to T_{EBV} (**see Section 15.J**).

STUDY DESIGN

6.A. Study Overview

The proposed study is a Phase I/II trial aimed at treating patients with AML who are at high risk for relapse after induction and standard consolidation chemotherapy. As previously described, most newly-diagnosed AML patients who initially respond to induction therapy will nevertheless ultimately relapse, mostly within the first 1-2 years. Furthermore, while it is generally accepted that HCT be considered as a potentially curative consolidative therapy for medically fit patients without favorable risk disease in first CR, many patients remain ineligible for HCT, either because of age, comorbidity, or lack of a suitable donor. Patients with detectable MRD at the time of CR, and patients with incomplete count recovery (CRi/CRp) represent a population at highest risk for disease relapse, particularly without allogeneic HCT and, thus, represent an appropriate target population for whom to consider an alternative strategy for reducing relapse. The estimated relapse rate in such a population is approximately 90% at 1 year.[21-23,47]

The proposed study will explore the safety and efficacy of adoptively transferred autologous T cells genetically modified to express a high affinity TCR that recognizes the WT1 antigen that is highly expressed in leukemic blasts. The high rate of relapse by 1 year that is observed without HCT suggests that we will be able to draw meaningful conclusions regarding the efficacy of our study intervention within only a few years of treating patients.[20,22-28]

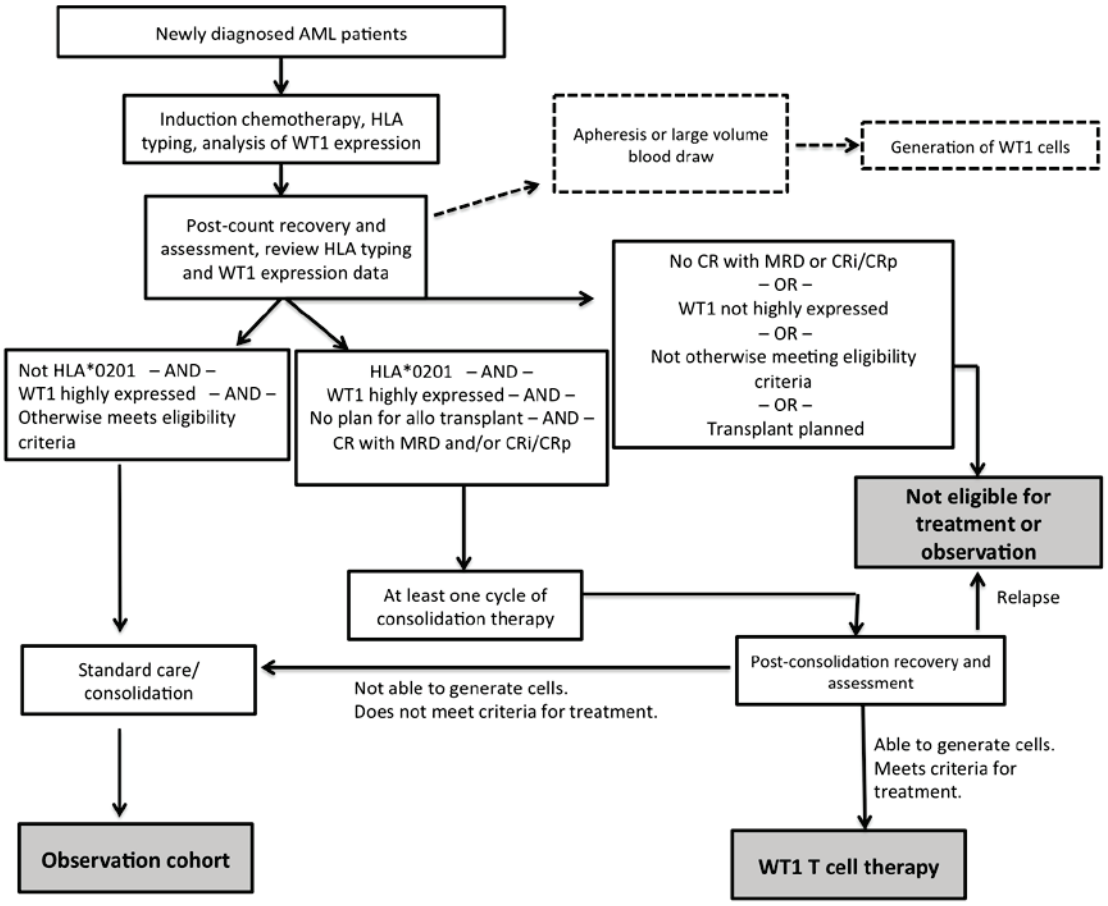
Newly diagnosed non-M3 AML patients who meet enrollment criteria (see **Section 7.A.)** will be enrolled on the study. Ideally, screening will be prior to initiation of induction therapy and enrollment will be prior to initiation of consolidation therapy, although patients will remain eligible for this study after treatment. Rapid HLA typing with PCR-based methods to determine the presence of HLA-A*0201 will be performed at the time of screening (unless HLA typing has already been completed). Leukemic cells will be assessed for expression of WT1in bone marrow or peripheral blood. Increased expression of WT1 will be determined by either of two methods: if

the number of copies of WT1 divided by the number of copies of ABL x 10^4 is > 250 for bone marrow, or > 50 for peripheral blood, based on the reported expression of WT1 in normal blood and marrow samples and the associated negative prognostic impact of increased levels[163]; or by immunohistochemical staining (IHC) in blasts as compared to adjacent normal myeloid and erythroid precursors, as determined by a Fred Hutchinson/Seattle Cancer Care Alliance pathologist. Patients with demonstrated increased expression of WT1 will be considered eligible for the study treatment or the observation cohort. Patients who are HLA-A*02:01 (confirmed by sequence analysis) will undergo leukapheresis or large volume blood draw after recovery of blood counts following systemic therapy.

The proposed study will aim to treat up to 35 AML patients at highest risk for relapse without HCT, as defined by presence of MRD by flow cytometry at time of remission and/or by incomplete recovery of neutrophil or platelet counts. We will assess the safety and feasibility of administering autologous WT1-specific T cells as post-remission AML therapy in this group.TCR_{C4} , for which our clinical experience is described in **Section 3.J.**, will also be used in this protocol. A contemporaneous cohort of similarly high risk patients who do not receive the study intervention, but who are also not candidates for HCT, will be followed prospectively to allow for an assessment of efficacy based on comparisons of relapse, DFS and OS.

Patients will undergo *at least* one cycle of consolidation therapy, at the discretion of their treating physician. After recovery, patients who meet treatment criteria (see **section 7.D.)** will receive the study intervention of infused autologous WT1-specific T cells. The time required to generate T cells is 15 to 20 days and, therefore, patients may go on to receive consolidation therapy. Given this logistical limitation in administering transduced T cells, and because several published reports highlight the adverse prognostic impact of MRD when detected by flow cytometry at the end of induction (prior to consolidation), otherwise-eligible patients who convert to an MRD-negative status during courses of consolidation therapy will remain eligible to receive the study treatment.[21-23,47,136,164] Patients who are not HLA-A*02:01 will be moved to the observation arm and treated per standard treatment plans or other clinical trials as per the discretion of their treating physician (**Figure 13**). Patients who undergo hematopoietic stem cell transplantation will be excluded from analysis in both the intervention arm and observation arm.

Figure 13. Study Schema



The proposed study will be conducted in two stages. In the first stage, we will sequentially treat three cohorts of 5 patients each with autologous TCR_{C4}-transduced T cells derived from: 1.) T_N and T_{CM} at a 1:1 ratio without conditioning chemotherapy; 2.) T_N and T_{CM} at a 1:1 ratio with lymphodepleting chemotherapy and 3.) T_N, T_{CM} and T_{EBV} cells at a 1:1:1 ratio with lymphodepleting chemotherapy. This approach is designed to determine the most promising plan of treatment with regards to safety, T cell persistence, and possibly efficacy. In stage 2, an additional 20 patients will be treated using what is determined to be the best/safest treatment

approach from stage 1, as described in **Section 23.A.2**. Because patients will receive a mix of polyclonal $CD8^+$ T_N and T_{CM} cells and T_{EBV} T cells transduced with TCR_{C4}, an aliquot of the transduced $CD8^+$ T_N and T_{CM} and T_{EBV} T cells will be taken for HTTCS analysis to characterize the composition of endogenous TCR genes, and allow distinction of each population during longitudinal monitoring following *in vivo* infusion. Monitoring of WT1/EBV bi-specific cells will also be based on the ability to bind WT1- and EBV-specific tetramers, which will additionally allow for determination of the subset origin of TCRC4-transduced cells derived from the EBV-specific subset.

6.B. Stage 1, Cohort #1

1. First infusion of WT1-specific T cells, cohort #1

In stage 1 of the study, 5 patients (cohort #1) will initially receive a 1:1 mix of polyclonal $CD8^+$ T_N and T_{CM} cells transduced with TCR_{C4} (**Figure 14)**. The infusions will be administered *without* lymphodepleting chemotherapy, consistent with the approach utilized in **FHCRC Protocol 2498**. Approximately 4 weeks after the last cycle of consolidation chemotherapy (depending on a given patient's clinical situation, preference of primary treating physician, and availability of the T cell product) patients will receive a first T cell infusion at a total target dose of 1×10^9 WT1-specific cells/m^2 (consisting of 0.5×10^9 each T_N and T_{CM}-derived cells). If the actual cell dose for either T cell subset is less than the target dose, or if cells from either subset cannot be generated, infusion of all available cells will be allowed.

2. Second infusion of WT1-specific T cells, cohort #1

The subsequent infusion will be held until the transferred cells persist at a level of < 5% of the total $CD8^+$ cells and at an absolute number of <30 cells/μl. Thus, after a minimum of 3 weeks, an additional (second) infusion may be given at a total target dose of 1×10^{10} WT1-specific cells/m^2 (consisting of 0.5×10^{10} each T_N and T_{CM}-derived cells).

3. IL-2 administration

The second infusion will be followed by low-dose s.c. IL-2 250,000 IU/m^2 twice daily x 14 days Patients or caregivers will be instructed on how to self-administer the drug. After the two planned infusions and IL-2 administration, patients will have completed the study treatment.

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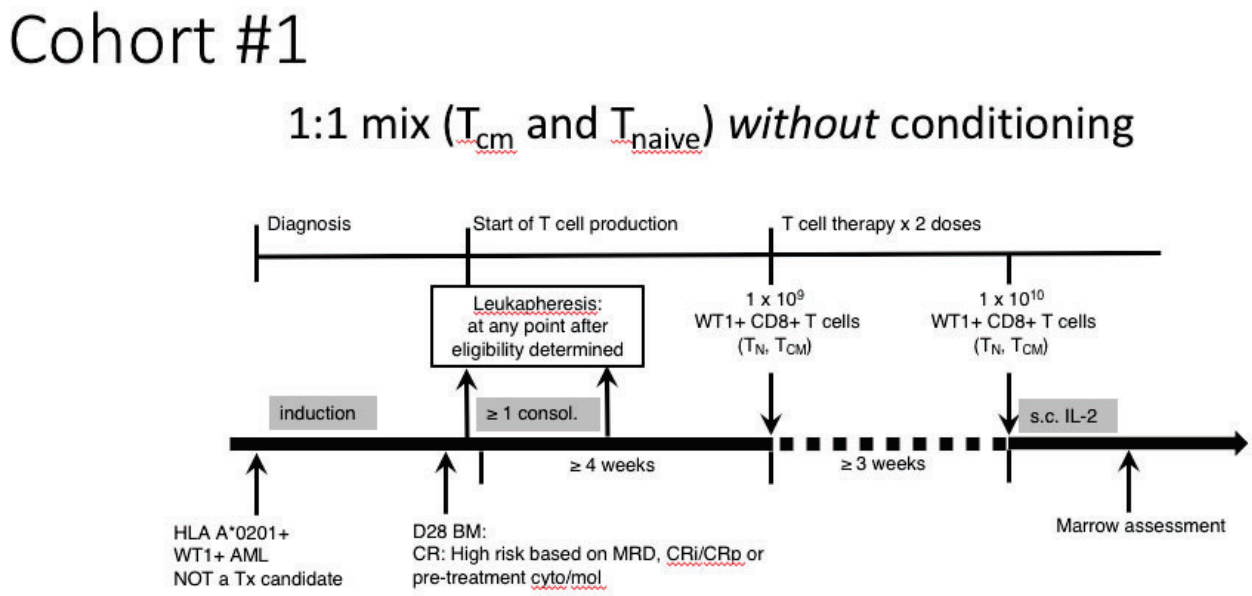
4. Timing of disease restaging with bone marrow sampling

Bone marrow evaluation will be performed within four weeks before the first infusion and approximately four weeks after the second infusion (approximately 14 days after completion of IL-2). Due to high persistence of infused T cells, however, the second infusion may be delayed for weeks to months. Therefore, if the interval between the first and second infusion is > 4 weeks, a repeat marrow evaluation will be performed at that time to assess disease response to the initial treatment. If the time interval between T cell infusions is ≥ 10 weeks, a repeat bone marrow evaluation will also be performed within four weeks prior to the second infusion, in order to allow for restaging prior to re-treatment.

Figure 14. Plan of Treatment (Stage 1, Cohort #1)



6.C. Stage 1, Cohort #2

1. First infusion of WT1-specific T cells, cohort #2

We will proceed with cohort #2 if the treatment intervention from the first cohort (administration of a 1:1 mix of polyclonal $CD8^+$ T_N and T_{CM} cells) is deemed to be acceptably safe, based on toxicity stopping rules as defined in **Section 23**. To assess whether T cell persistence (and possibly efficacy) is augmented with lymphodepleting chemotherapy, the 5 patients in cohort #2 will be treated with a course of lymphodepleting chemotherapy prior to receiving a 1:1 mix of polyclonal $CD8^+$ T_N and T_{CM} cells transduced with TCR_{C4} (**Figure 15)**. The suggested lymphodepletion regimen will consist of cyclophosphamide (300 mg/m2) and fludarabine (30 mg/m^2) daily for 3 days (days -4 to -2). Dose reductions or omission of chemotherapy will be allowed as per the patient's clinical situation and discretion of the PI or treating attending physician.

Patients will begin the conditioning regimen approximately 4 weeks after the last cycle of consolidation chemotherapy (depending on the patient's clinical situation, the preference of primary treating physician, and the availability of the T cell product). TCR_{C4}-transduced T cells will be infused at 36-96 hours after the last dose of chemotherapy, though delayed infusion of T cells at > 96 hours post-conditioning will be allowable if there are clinical or logistical issues preventing infusion in the specified window. Patients will receive a total target dose of 1 x 10^9 WT1-specific cells/m^2 (consisting of 0.5 x 10^9 each T_N and T_{CM}-derived cells). If the actual cell dose for either T cell subset is less than the target dose, or if cells from either subset cannot be generated, infusion of all available cells will be allowed.

2. Second infusion of WT1-specific T cells, cohort #2

As with cohort #1, the subsequent infusion will be held until the transferred cells persist at a level of < 5% of the total $CD8^+$ cells and at an absolute number of <30 cells/µl. Thus, after a minimum of 3 weeks, an additional (second) infusion may be given at a total target dose of 1 x 10^{10} WT1-specific cells/m^2 (consisting of 0.5 x 10^{10} each T_N- and T_{CM}-derived cells). If the actual cell dose for any T cell subset is less than the target dose, or if cells from either subset cannot be generated, infusion of all available cells will be allowed. As with the first infusion, patients will again be treated with a course of lymphodepleting chemotherapy prior to receiving a 1:1 mix of polyclonal $CD8^+$ T_N and T_{CM} cells transduced with TCR_{C4}. The suggested lymphodepletion regimen will consist of cyclophosphamide (300 mg/m2) and fludarabine (30 mg/m^2) daily for 3

days (days -4 to -2). Dose reductions in chemotherapy will be allowed as per the patient's clinical situation and discretion of the PI or treating attending physician. While it may seem counterproductive to administer chemo-therapy prior to the second planned infusion, when up to 5% of CD8+ cells may be WT1-specific, this study design strategy will allow the opportunity to evaluate persistence and efficacy at the 1.0 x 10^9 cells/m^2 dose level, potentially rendering obsolete the higher dose infusion of 1.0 x 10^{10} cells/m^2 which is associated with considerably longer production times and higher treatment costs.

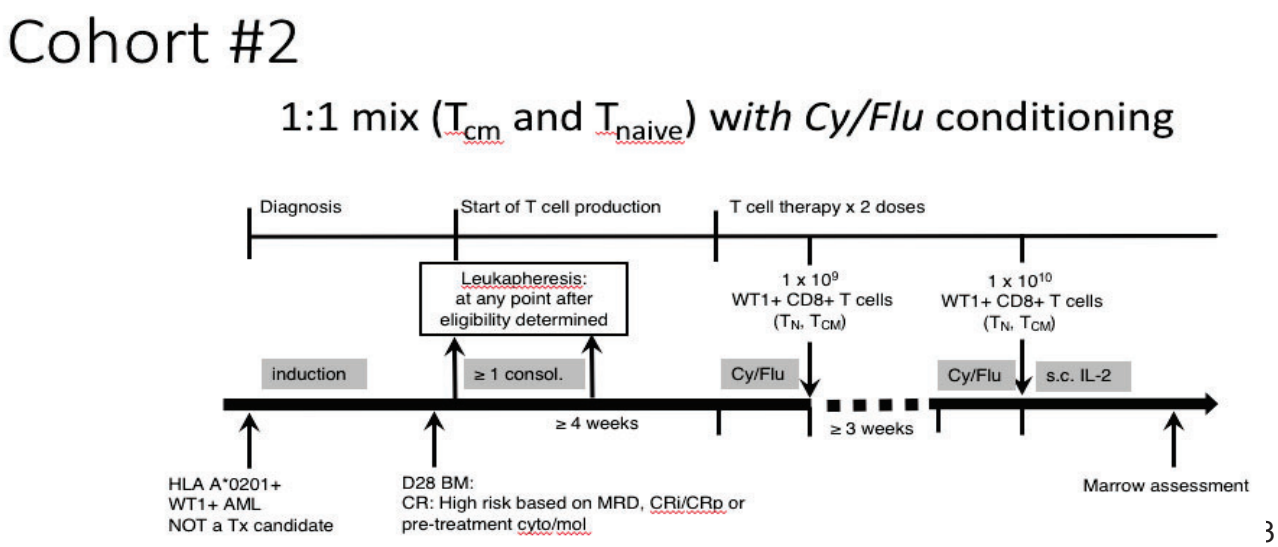
3. IL-2 administration

The second infusion will be followed by low-dose s.c. IL-2 at 250,000 IU/m^2 twice daily x 14 days. After the two planned infusions and IL-2 administration, patients will have completed the study treatment.

4. Timing of disease restaging with bone marrow sampling

Bone marrow evaluation will be performed within six weeks prior to the first T cell infusion and approximately four weeks after the second infusion (approximately 14 days after completion of IL-2). Due to high persistence of infused T cells, however, the second infusion may be delayed for weeks to months. Therefore, if the interval between the first and second infusion is > 4 weeks, a repeat marrow evaluation will be performed at that time to assess disease response to the initial treatment. If the time interval between T cell infusions is ≥ 10 weeks, a repeat bone marrow evaluation will also be performed within four weeks prior to the second round of lymphodepleting chemotherapy and second infusion, in order to allow for restaging prior to re-treatment.

Figure 15. Plan of Treatment (Stage 1, Cohort #2)



6.D. Stage 1, Cohort #3

1. First infusion of WT1-specific T cells, cohort #3

We will proceed with the third cohort of patients if the treatment intervention, including lymphodepleting chemotherapy, is deemed to be acceptably safe, based on toxicity stopping rules as defined in **Section 23**. To assess whether the long-term persistence of WT1-specific T cells on FHCRC Protocol 2498 is possibly dependent on the infusion of modified T cells that are EBV-specific, such that the persistence could reflect exposure to EBV antigen, the next 5 patients (cohort #3) will receive a 1:1:1 mix of polyclonal $CD8^+$ T_N and T_{CM} and T_{EBV} T cells transduced with TCR_{C4}, *with* lymphodepleting chemotherapy (**Figure 16)**. The suggested lymphodepletion regimen will consist of cyclophosphamide (300 mg/m2) and fludarabine (30 mg/m^2) daily for 3 days (days -4 to -2). Dose reductions in chemotherapy will be allowed as per the patient's clinical situation and discretion of the PI or treating attending physician.

As with cohort #2, patients will begin the conditioning regimen approximately 4 weeks after the last cycle of consolidation chemotherapy (depending on the patient's clinical situation, the preference of primary treating physician, and the availability of the T cell product). TCR_{C4}-transduced T cells will be infused at 36-96 hours after the last dose of chemotherapy, though delayed infusion of T cells at >96 hours post-conditioning will be allowable if there are clinical or logistical issues preventing infusion in the specified window. Patients will receive a total target dose of 1.5 x 10^9 WT1-specific cells/m^2 (consisting of 0.5 x 10^9 each T_N and T_{CM}-derived cells and T_{EBV} cells). If the actual cell dose for any T cell subset is less than the target dose, or if cells from any subset cannot be generated, infusion of all available cells will be allowed.
If the addition of lymphodepleting chemotherapy in cohort #2 is associated with unacceptable toxicity, as defined in **Section 23**, cohort #3 will proceed with a 1:1:1 mix of polyclonal $CD8^+$ T_N and T_{CM} and T_{EBV} T cells transduced with TCR_{C4} *without* lymphodepleting chemotherapy.

2. Second infusion of WT1-specific T cells, cohort #3

As with the previous cohorts, the subsequent infusion will be held until the transferred cells persist at a level of <5% of the total $CD8^+$ cells and at an absolute number of <30 cells/µl. Thus, after a minimum of 3 weeks, an additional (second) infusion may be given at a total target dose of 1.5 x

10^{10} WT1-specific cells/m^2 (consisting of 0.5 x 10^{10} each T_N- and T_{CM}-derived cells and T_{EBV} cells). If the actual cell dose for any T cell subset is less than the target dose, or if cells from either subset cannot be generated, infusion of all available cells will be allowed. As with the first infusion, patients will again be treated with a course of lymphodepleting chemotherapy prior to receiving a 1:1:1 mix of polyclonal $CD8^+$ T_N and T_{CM} and T_{EBV} T cells transduced with TCR_{C4}. The suggested lymphodepletion regimen will consist of cyclophosphamide (300 mg/m2) and fludarabine (30 mg/m^2) daily for 3 days (days -4 to -2). Dose reductions in chemotherapy will be allowed as per the patient's clinical situation and discretion of the PI or treating attending physician.

3. IL-2 administration

The second infusion will be followed by low-dose s.c. IL-2 at 250,000 IU/m^2 twice daily x 14 days. After the two planned infusions and IL-2 administration, patients will have completed the study treatment.

4. Timing of disease restaging with bone marrow sampling

Bone marrow evaluation will be performed within four weeks of the initiation of lymphodepleting chemotherapy given immediately prior to T cell infusion, and approximately four weeks after the second infusion (approximately 14 days after completion of IL-2). Due to high persistence of infused T cells, however, the second infusion may be delayed for weeks to months. Therefore, if the interval between the first and second infusion is > 4 weeks, a repeat marrow evaluation will be performed at that time to assess disease response to the initial treatment. If the time interval between T cell infusions is ≥ 10 weeks, a repeat bone marrow evaluation will also be performed within four weeks prior to the second round of lymphodepleting chemotherapy and second infusion, in order to allow for restaging prior to re-treatment.

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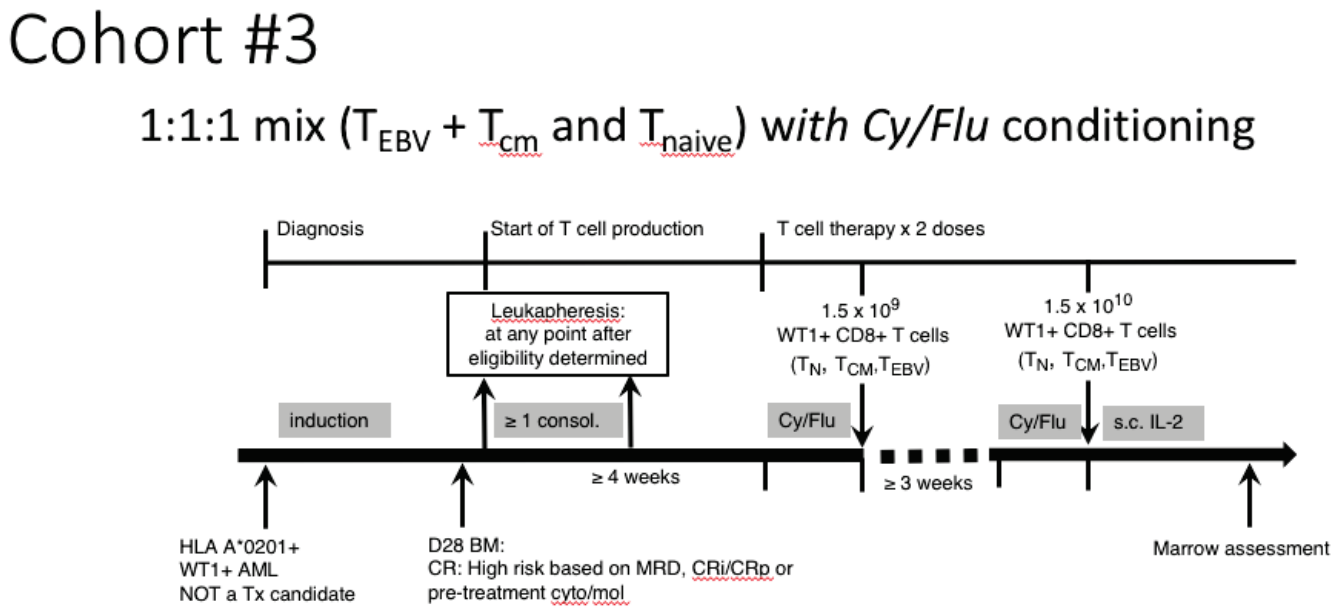
Figure 16. Plan of Treatment (Stage 1, Cohort #3)



6.E. Stage 2

After 15 patients (across 3 study cohorts in stage 1) have received at least 1 infusion each, and if the study treatment has been shown to be safe (as outlined in the statistical plan, **Section 23**), we will start enrolling patients on Stage 2 of the proposed study. The study team will convene with the Data Safety Monitoring Board in order to determine the most promising plan of treatment with regards to safety, T cell persistence, and efficacy (again, as outlined in the statistical plan, **Section 23**). In stage 2, an additional 20 patients will be treated using the selected treatment approach, for a planned total of 35 patients treated.

1. First infusion of WT1-specific T cells, Stage 2

All patients will receive a mix of TCR_{C4}-transduced cells derived from polyclonal $CD8^+$ T_N and T_{CM} +/- T_{EBV} T cells, as determined upon review of safety, T cell persistence and potentially available efficacy data from Stage 1, as outlined in **Section 23.A.2**. Unless determined to be unacceptably safe in Stage 1, lymphodepleting chemotherapy will be administered prior to T cell therapy, consisting of cyclophosphamide and fludarabine, as detailed above in stage 1.

Patients will begin the conditioning regimen approximately 4 weeks after the last cycle of consolidation chemotherapy (depending on the patient's clinical situation, the preference of primary treating physician, and the availability of the T cell product). TCR_{C4}-transduced T cells will be infused at 36-96 hours after the last dose of chemotherapy, though delayed infusion of T cells at >96 hours post-conditioning will be allowable if there are clinical or logistical issues preventing infusion in the specified window. Patients will receive a total target dose of 1.0 – 1.5 x 10^9 WT1-specific cells/m^2 (consisting of a mix of TCR_{C4}-transduced cells derived from polyclonal $CD8^+$ T_N and T_{CM} +/- T_{EBV} T cells, as determined by the study team and DSMB). If the actual cell dose for any T cell subset is less than the target dose, or if cells from any subset cannot be generated, infusion of all available cells will be allowed.

2. Second infusion of WT1-specific T cells, Stage 2

The subsequent infusion will be held until the transferred cells persist at a level of <5% of the total $CD8^+$ cells and at an absolute number of <30 cells/µl. Thus, after a minimum of 3 weeks, an additional (second) infusion may be given at a total target dose of 1.0 – 1.5 x 10^{10} WT1-specific cells/m^2 (consisting of a mix of TCR_{C4}-transduced cells derived from polyclonal $CD8^+$ T_N and T_{CM} +/- T_{EBV}) T cells, as determined by the study team and DSMB). If the actual cell dose for any T cell subset is less than the target dose, or if cells from either subset cannot be generated, infusion of all available cells will be allowed.

3. IL-2 administration

Low-dose s.c. IL-2 at 250,000 IU/m^2 twice daily x 14 days will follow the 2^{nd} infusion. After the two planned infusions and IL-2 administration, patients will have completed the study treatment.

4. Timing of disease restaging with bone marrow sampling

Bone marrow assessment will similarly be performed as described above in **Section 6B.**

PATIENT SELECTION

7.A. Eligibility for Enrollment

1. Patients with (non-M3) acute myeloid leukemia (AML).

2. Patients must be ≥15kg, as patients with lower weight would be incapable of providing high volume and frequent blood samples for monitoring and analysis.
3. Patients or parents/legal guardian must be able to give informed consent.
4. Patients must be able to provide blood and marrow samples and to undergo the procedures required for this protocol.
5. Elevated expression of WT1 in pre-treatment bone marrow or peripheral blood by either of two methods:
    a. Increased expression of WT1 determined if the number of copies of WT1 divided by the number of copies of ABL x 10^4 is > 250 for bone marrow, or > 50 for peripheral blood.[163];
    b. Demonstration of WT1 overexpression will be determined by immunohistochemical staining (IHC) in blasts as compared to adjacent normal myeloid and erythroid precursors, as determined by a Fred Hutchinson/Seattle Cancer Care Alliance pathologist.
6. Demonstration of disease response to induction chemotherapy, in that patients must have achieve a morphologic remission (marrow that is at least 10% cellular with < 5% blasts on morphologic review) after 1-2 induction cycles, regardless of minimal residual disease or CRi/CRp status.
7. Determination of “high-risk” disease. Subjects must meet one of the determinants of “high-risk disease”, in terms of being at very high risk for relapse without allogeneic stem cell transplant, as per one of the follow criteria:
    a. A designation of “adverse” risk disease at the time of diagnosis, as defined by cytogenetic and molecular abnormalities specifically outlined in the 2017 European LeukemiaNet (ELN) guidelines for diagnosis and management of AML.[142] These patients will meet “high-risk” designation, regardless of minimal residual disease or CRi/CRp status.
    b. Relapsed leukemia. Patients with cytogenetic or molecular classification other that adverse risk by ELN who go on to demonstrate disease relapse after a minimum duration of remission of 6 months, but who then attain a second complete remission with repeat induction chemotherapy, as defined above in **Section 7.A.6**. These patients will meet “high-risk” designation, regardless of minimal residual disease or CRi/CRp status.

c. Minimal residual disease, as defined by having detectable disease by one of the following criteria , but otherwise being in morphologic remission, **as per Section 7.A.6**.
    i. MRD by flow cytometry at any time after induction chemotherapy or during consolidation chemotherapy, when patients are otherwise classified as being in morphologlic remission **as per Section 7.A.6**, and as defined by any abnormal myeloid blasts identified by flow cytometric analysis.
    ii. Cytogenetic MRD, as defined by a disease-specific abnormal karyotype at any point in patients who are otherwise in morphologic remission, **as per Section 7.A.6**
    iii. Molecular minimal residual disease (MRD) with one of the following markers, as specified below, in patients who are otherwise in morphologic remission, **as per Section 7.A.6**:
        1. a normalized copy number (NCN) of > 0.001 for *CBFB/MYH11* or a normalized copy number (NCN) of > 0.050 for *AML1/ETO (RUNX1/RUNX1T1) after at least 4 cycles of consolidation chemotherapy*
        2. a < 2 log reduction in either *CBFB/MYH11* or *AML1/ETO (RUNX1/RUNX1T1)* in the bone marrow at the time of post-induction disease restaging (immediately after 1-2 cycles of induction therapy)

d. CRi/CRp, as defined by neutrophil count < 1000/µl (CRi) and/or platelet count < 100,000/µl (CRp), but otherwise being in morphologic remission, **as per Section 7.A.6**. In pediatric patients, a platelet threshold of < 80,000/ µl will be used, as per consensus pediatric response criteria.[76]

8. HLA-A*02:01 expression must be present for patient to be on treatment arm, HLA*A02:01 expression absent in patients designated to observation arm.

7.B. Exclusion Criteria for Enrollment

1. Active autoimmune disease (e.g. systemic lupus erythematosus, vasculitis, infiltrating lung disease, inflammatory bowel disease) in which possible progression during treatment would be considered unacceptable by the investigators.
2. Previous allogeneic HCT.

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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. Pregnant women, nursing mothers, men or women of reproductive ability who are unwilling or unable to use effective contraception or abstinence. Women of childbearing potential must have a negative pregnancy test within two weeks prior to enrollment and initiation of treatment.
5. Clinically significant and ongoing immune suppression including, but not limited to: systemic immunosuppressive agents such as cyclosporine or corticosteroids (at an equivalent dose of 0.5 mg prednisone/kg per day, or higher), chronic lymphocytic leukemia (CLL), uncontrolled human immunodeficiency virus (HIV) infection (untreated or detectable viral load within 3 months of enrollment).
6. Acute promyelocytic leukemia (M3 leukemia, per French-American-British classification)

7.C. Eligibility for Apheresis/blood collection

1. HLA-A*02:01 expression

7.D. Eligibility for Treatment with TCR_{C4}-transduced $CD8^+$ cells

1. Response to therapy and completion of ***at least one*** cycle of consolidation therapy, and with disease status meeting one of the aforementioned “high-risk” criteria at the time of post-induction disease restaging as already outlined in **Section 7.A.6**, above.
2. Hematologic recovery from induction and other post-remission therapy (ANC > 200/µl, platelet count > 20,000/µl) at the time of the study intervention
3. No plan for allogeneic stem cell transplantation within 3 months.
4. Elevated expression above baseline of WT1 in bone marrow or peripheral blood.
5. Additionally, patients treated in stage 1, cohort #3 must be EBV seropositive, given the inclusion of T cells derived from an EBV-specific subset in this group
6. Continued morphologic remission (<5% blasts in the marrow, no circulating blasts or known extramedullary relapse) within 6 weeks of receiving the study intervention (specified as T cell infusion for cohort 1, or the start of lymphodepleting chemotherapy for cohorts 2 and 3).

7.E. Exclusion for Treatment with TCR_{C4}-transduced $CD8^+$ cells

1. Unable to generate antigen-specific WT1-specific $CD8^+$ T cells for infusions. However, if a lower than planned number of cells is available, the patient will have the option to receive the generated WT1-specific T cells.
2. Systemic steroids should be stopped 2 weeks before the start of treatment. Topical and inhaled steroids are allowed.
3. Symptomatic and refractory central nervous system (CNS) leukemia.
4. Complete blood count (CBC) profile prior to treatment:
   - Absolute neutrophil count (ANC) <200/µl
   - Platelets <20,000/µl

   If a patient meets other treatment eligibility but otherwise demonstrates delayed or poor recovery of peripheral blood counts to the above neutrophil and/or platelet thresholds, then treatment with the T cell intervention will be allowed if:

   a) Neutrophil and/or platelet counts remain below the thresholds after a period of at least 6 weeks from last systemic chemotherapy; OR neutrophil and/or platelet counts remain below the thresholds in the setting of a maintenance therapy, such as midostaurin); and
   b) The patient has detectable leukemia (e.g. flow cytometry positive or MRD by FISH or molecular testing); and
   c) The P.I. or treating physician documents that the likely cause of cytopenias is underlying disease as opposed to another cause (e.g. medication).

5. Ongoing ≥ grade 3 cardiac, pulmonary, renal, gastrointestinal or hepatic toxicities according to NCI CTCAE version 4 toxicity criteria.
6. Karnofsky performance status score (age ≥16 years) or Lansky play score (age <16 years) ≤40%. (**Appendix A**)
7. Medical or psychological conditions that, according to the PI, would make the patient unsuitable candidate for cell therapy.
8. Pregnancy or breast-feeding. Women of childbearing potential must have a negative serum or urine β-hCG pregnancy test result within 14 days before the first dose of WT1-specific T cell infusions. Woman of non-childbearing potential will be defined as being postmenopausal greater than one year or who have had a bilateral tubal ligation or hysterectomy. All recipients of WT1-specific T cells will be counseled to use effective birth control during participation in this study and for 12 months after the last T cell infusion.
9. Treatment with alemtuzumab or other T cell-depleting antibodies within 6 months of T cell therapy.

10. Documented new infection within 24 hours of T cell infusion, or concern for new infection as suggested by an oral temperature >38.2°C within 24 hours of T cell infusion.
    a. In patients who have T cells delayed because of development of fever (oral temperature >38.2°C) and who subsequently become afebrile (38.2°C or less) for 24 hours without a documented infection, T cells may be administered.
    b. In patients with a documented new infection within 24 hours of planned T cell infusion, they may go on to receive T cells after administration of directed antibiotic therapy and if they subsequently remain afebrile (38.2°C or less) for at least 24 hours, and if it is deemed clinically appropriate by the PI.
    c. Pre-existing infections requiring chronic maintenance therapy (e.g. chronic HBV or treated bacterial infections) are not an exclusion for T cell infusion as long as patients are on appropriate antimicrobial therapy for at least 1 month (e.g. for chronic hepatitis B or C viral infection) and who remain afebrile and without symptomatic evidence for uncontrolled chronic infection within 24 hours of T cell infusion. Patients should also have a negative HIV test by viral load within 3 months of treatment.

7.F. Eligibility for Observation Arm

1. The patient meets all of the eligibility criteria for enrollment, as per **Sections 7.A and 7.B**, but lacks expression of HLA-A*0201 as is needed to be enrolled on the treatment arm and for apheresis, as per **Sections 7.A and 7.C.**

CONSENTING

A consent conference will be held with eligible patients. The PI or a delegated representative will discuss this study and alternative treatments available for AML. All known risks and potential hazards of treatment with WT1-specific transduced memory or 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 FHCRC Institutional Review Board (IRB). After the consent is signed, the patients' HLA-A type will be evaluated and leukemia cells will be evaluated for WT1 expression. HLA-A*02:01^+ patients with elevated WT1 expression will undergo a leukapheresis or large volume peripheral blood collection to obtain peripheral blood mononuclear cells (PBMC) used to

generate the cellular product. Patients will be moved to the observation arm of the study if they do not express HLA-A*02:01, if they do not meet treatment criteria (as defined in **sections 7D and 7E**), or if WT1-specific T cells cannot be generated.

PROTOCOL REGISTRATION

Patients will be assigned to the protocol by the Trial Coordinator who will register the patients 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.

PROCEDURE TO OBTAIN PBMC FOR GENERATION OF THERAPEUTIC T CELLS

The preferred source of PBMCs will be a non-granulocyte colony-stimulating factor (GCSF)-mobilized 6 or 12 liter leukapheresis which will be performed after determination of eligibility and recovery from cytoreductive therapy. 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 may include:

- Active infection.
- Recent hepatitis exposure, hepatitis A or B antigenemia, or hepatitis C antibody positivity.
- Pregnancy or nursing.
- HIV or HTLV infection.

Leukapheresis (or peripheral blood draw) for collection of autologous T cells may be repeated if there are an insufficient number of T cells available for either the first or second (higher dose) infusion of modified T cells, as determined by the PI/study team and with the consent of the patient.

If the patient is unable or unwilling to undergo leukapheresis, adults may alternatively have 120-200 mL of peripheral blood drawn for generation of the T cells. Children < 18 years old will have at least 50 mL of peripheral blood drawn for generation of the T cells, based on weight. If weight

does not allow for 50 mL of peripheral blood, then the patient must be excluded as this would compromise our ability to generate a product. PBMC may be cryopreserved and used to generate T cells for this study.

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 AML, contain autologous transduced WT1-specific CD8$^+$ T cells derived from T_{CM} and T_N cells, 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 will be further qualified using mycoplasma, fungal, and bacterial sterility testing.

HANDLING OF T CELL PRODUCTS BEFORE INFUSION

For each infusion dose, the gene-modified cell product will be formulated at the desired cell dose in a final volume of 75 - 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 research nurse or designee will then administer the cells to the patient over 1 to 4 hours (as described in **Section 14.C.).**

OTHER STUDY AGENTS

13.A. Interleukin-2

IL-2 will be initiated within 6 hours of the second T cell infusion (second T cell infusion at a dose of 1.0 x 1010 cells/m2), at a dose of 250,000 U/m^2 s.c. twice daily x 14 days. The patient or the caregiver will be instructed on s.c. self-administration.

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13.B. Cyclophosphamide

CY 300 mg/m^2 will be administered intravenously daily for 3 total doses (on days -4 to -2) prior to administration of T cells. Standard practice policy guidelines will be followed, and its administration will be completed at least 36 hours prior to the T cell infusion. Dose reductions will be allowed as per the patient's clinical situation and discretion of the PI or treating attending physician.

13.C. Fludarabine

Fludarabine at 30 mg/m^2 will also be administered daily for 3 total doses (on days -4 to -2) prior to administration of T cells. Its administration will be completed at least 36 hours prior to the T cell infusion. Dose reductions will be allowed as per the patient's clinical situation and discretion of the PI or treating attending physician.

PLAN OF TREATMENT

14.A. Screening Consent

After signing the informed consent for screening, patients will be screened for their eligibility, and HLA-A typing and analysis of WT1 expression will be performed. If the patient is HLA-A*02:01+ and has high WT1 expression, a leukapheresis (or large volume blood draw) may be scheduled after recovery of patients' counts after chemotherapy. Patients who do not express HLA-A*02:01 but who otherwise meet study criteria will be moved to the "observation arm" of the study. Those found to be eligible for treatment will be consented for the study during a subsequent consent visit. Pediatric patients who consent to the study will continue to be treated by the pediatric Hematology/Oncology service at Seattle Children's, whereas it is planned that adult patients who consent to the study will be managed by the Immunotherapy service at the Seattle Cancer Care Alliance.

14.B. Leukapheresis Visit

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Patients will undergo leukapheresis following cytoreductive chemotherapy. Adult patients will preferably undergo the leukapheresis procedure in the outpatient setting (5th floor Apheresis Unit) at the Seattle Cancer Care Alliance, unless clinical circumstances dictate that leukapheresis must be done as an inpatient at the University of Washington Medical Center. Pediatric patients will undergo the leukapheresis procedure at Seattle Children's (Ambulatory Nursing Infusion Unit or inpatient setting, if clinically indicated). Leukapheresis will be performed after recovery from treatment (ANC >500/µl, platelet > 30,000/µl) or alternatively a large volume blood draw will be performed, as outlined in **Section 10**. Separate consent for the leukapheresis procedure will be obtained, as per Seattle Cancer Care Alliance, University of Washington Medical Center, and Seattle Children's policies.

14.C. Lymphodepletion Chemotherapy

Patients who enroll to be treated on the study, other than the initial 5 patients treated on cohort #1, will receive lymphodepleting conditioning chemotherapy as outlined in the Study Design (**Section 6**). Pediatric patients meeting treatment eligibility will receive chemotherapy under the continued care of the pediatric Hematology/Oncology service at Seattle Children's (Ambulatory Nursing Infusion Unit). It is planned that adult patients meeting treatment eligibility will receive chemotherapy in the outpatient setting (Immunotherapy Clinic or 5th floor Infusion Unit) at the Seattle Cancer Care Alliance under the care of the Immunotherapy service.

14.D. T Cell Infusion

For adults, T cell infusions will be given in the outpatient setting (Immunotherapy Clinic or 5th floor Infusion Unit) at the Seattle Cancer Care Alliance, or alternatively at the University of Washington (UW) if the study subject is an inpatient at the UWMC yet meets criteria for treatment (**Sections 7D and 7E**) at the time of T cell infusion. Adult patients may also be admitted for infusion and overnight observation at the Translational Research Unit (TRU) at the UWMC, at the discretion of the study team. Pediatric patients will receive T cell infusions in the Ambulatory Nursing Infusion Unit located at Seattle Children's or alternatively in the inpatient setting at Seattle Children's if the study subject is an inpatient yet meets criteria for treatment (**Sections 7D and 7E**) at the time of T cell infusion. For adults, T cells will be infused intravenously at a rate of 250 cc/hour through an 18- or 20-gauge catheter inserted into a peripheral vein or through a central catheter. For children, T cells will be infused intravenously over 1-4 hours through an 18- or 20-gauge catheter inserted

into a peripheral vein or through a central catheter. The infusion bag will be gently mixed during the infusion. Subjects will have vital signs and oxygen saturation measured by pulse oximetry obtained at the start of the infusion, every 15 minutes during the infusion, at the end of the infusion, and hourly for 2 hours following the infusion. Intravenous fluids will be administered post-infusion at the discretion of the PI or treating physician. If no adverse events requiring more intensive monitoring or continued hospitalization have occurred, the patients will be released two hours following the infusion or at the completion of prescribed intravenous fluids, whichever is later (see **Section 16**). Patients will contact the clinic or overnight medical provider with any fever > 100.5^{o}C or other concerning symptoms in the post-infusion period, and will be triaged according to standard clinical practice.

14.E. Low-dose S.C. IL-2

After cell infusions of 1.0 x 10^{10} cells/m^2, patients will receive twice daily s.c. injections of recombinant IL-2 at a dose of 2.5 x 10^5 U/m^2 every 12 hours for 14 days (28 doses) with the first dose starting between 2-4 hours after completing the T cell infusion. Patients and/or caregivers 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.

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 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.

15.A. Patient Evaluation during the Treatment Consent Visit

Patients will have had a history taken, a physical exam and blood draw performed. Results of previous marrow examination will be reviewed. Eligibility for enrollment will be checked.

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15.B. Patient Evaluation at Each Planned Visit

Patients will have a history taken, a physical exam with vital signs performed, and a comprehensive chemistry panel and complete blood count obtained at planned visits including prior to the T cell infusion, 1 and 7 days after each infusion, and weekly up to 4 weeks after each infusion; urinalysis will be obtained prior to the T cell infusion, and at 14 and 28 days after each infusion (**Appendix B**), or as otherwise clinically indicated.

15.C. Evaluation of WT1 expression by leukemic cells

A two mL (in EDTA) aliquot for research will be obtained with each bone marrow aspirate the patient undergoes after enrollment. If the patient has circulating leukemic blasts in peripheral blood, 10 mL (in EDTA) of peripheral blood may also be obtained at time of enrollment (pre-treatment). WT1 expression will be assessed by PCR or by immunohistochemical (IHC) staining in pre-treatment peripheral blood or bone marrow samples. Peripheral blood and bone marrow specimens may also be collected at selected time points following therapy, as per **Section 15.G,** for analysis of WT1 expression in residual or relapsed leukemic cells.

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 prior to initiation of infusion, every 15 minutes during the 1 to 4 hour T cell infusion, and then hourly for 2 hours following the T cell infusion. AEs will be managed by standard medical practice (see **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 day of lymphodepletion chemotherapy (or in those patients in stage 1, cohort 1 who do not receive chemotherapy such monitoring will start with the first T cell infusion), and continue for 3 months after each infusion, as outlined in **Section 15.E.1**. Subsequently, patients will continue to be monitored for evidence of long term effects of treatment with lentivirus-transduced T cells on at least an annual basis, as outlined in our plan for Long Term Follow-up in **Section 15.K**. The

evaluation days listed in **Appendix B** are approximate and it is anticipated that patients who reside outside the Seattle area would not always be able to follow the exact time points as dictated by the protocol. In addition, frequency and duration of monitoring may be increased if indicated based on the patient's clinical condition and/or duration of cells persistence.

15.E.1 General Toxicity Assessment

All Grade ≥3 Adverse Events (AEs) will be recorded and graded according to the NCI CTCAE v4.0 up to 4 weeks after each infusion. Thereafter, unexpected, related and serious adverse events (SAEs) will be recorded through the end-of-study (see **Section 15.I.)**. To evaluate for potential toxicities, patients will undergo a history and physical exam and laboratory evaluations (comprehensive chemistry panel and complete blood count) immediately prior to infusions, and on days +1, +7, +14, +21 and +28, and at 2 months and 3 months after each infusion; urinalysis will be obtained prior to the T cell infusion, and at 14 and 28 days after each infusion. Although the AE reporting period is designated to end at 4 weeks following each given T cell infusion, the study team will attempt to obtain and review any internal or external clinical or laboratory records at approximately 2 and 3 months post T cell infusion, and then at quarterly intervals thereafter, in order to monitor for any SAEs that might develop outside the specified AE reporting period. Additionally, the persistence of T cells will be assessed every 3 months. Following the last infusion, patients will be monitored, as above. Adjustment of the timing of toxicity assessment may be indicated per patient's clinical situation and at the discretion of the PI or designee.

Monitoring for treatment-related toxicity will be discontinued if a patient becomes eligible and undergoes allogeneic HCT, or if the patient receives other systemic therapy for AML.

15.F. Feasibility Assessment (Primary Endpoint)

All screened patients will be assessed for expression of HLA A*0201, degree of WT1 expression cytogenetic and molecular features, and burden of disease following induction and consolidation therapy. In eligible patients, the proportion of those for whom a T-cell product can be generated from PBMC and time required to generate the product will be tracked. The feasibility of administering autologous WT1-specific T cells as post-remission AML therapy will be evaluated as:

1. The proportion of subjects who undergo leukapheresis or large volume blood draw who are ultimately determined to be eligible to receive T-cells.

2. The proportion of subjects who sign the treatment consent and undergo a leukapheresis or large volume blood draw, from which we are and are not able to generate a product.
3. The proportion of subjects who sign the treatment consent, have a T cell product generated, and ultimately receive the study intervention.

15.G. Evaluation of Persistence and/or Efficacy of Adoptively Transferred T Cells (Primary Endpoint)

15.G.1 Collection of Blood Sample

For adults and children >50kg, 60 ml of blood with heparin (green top) and 10 ml of blood in a serum separator (gold top) will be drawn prior to infusions (including at the time of screening and/or prior to treatment with the study intervention) and at selected time points following each infusion per **Section 15.E** (see also **Appendix B**, blood draw for research). The pre-specified time points are approximate, with some flexibility due to patient condition or scheduling, and the PI may request additional research specimens in the interval between infusions as is necessary to determine the persistence level of residual WT1-specific T cells and therefore eligibility for the subsequent infusion. The PI may also request additional blood samples for toxicity monitoring or persistence testing during the Long Term Follow Up period, as clinically indicated. For children weighing 30-50kg, 30 ml of blood with heparin (green top) and 5 ml of blood in a serum separator (gold top) will be drawn; and for children weighing 15-30kg, 1 ml/kg of blood with heparin (green top) and 3 ml of blood in a serum separator (gold top) will be drawn per schedule. All blood samples will be kept at room temperature and sent to FHCRC Research Cell Bank, Eastlake Building, E1-305. An aliquot of the blood collected will be used to evaluate *in vivo* persistence of infused T cells, including the derivation from the T_N and T_{CM} subsets (see **Section 15.G.3**).

15.G.2 Collection of Bone Marrow and Extramedullary Samples

Patients will undergo bone marrow evaluation within 6 weeks prior to the first T cell infusion to evaluate pre-treatment disease status and endogenous WT1 specific CTL responses, and also approximately 14 days after completion of IL-2 to assess disease response to the treatment intervention, or as clinically indicated per the treating physician. If high persistence of infused T cells is detected (≥5% of CD8+ cells), resulting in delay of the second infusion ≥ 4 weeks, a repeat marrow evaluation may be performed to assess disease response to the initial treatment. If the time interval between T cell infusions is ≥ 10 weeks, a repeat bone marrow evaluation will also be performed within six weeks prior to the second infusion, in order to allow for restaging prior to

re-treatment. Bone marrow evaluation in addition to, or outside of, these specified time points will be allowed as clinically indicated, as per the patient's treating physician.
Bone marrow samples, as per the approved methodk may be also obtained, if patients have signed screening consent 9296R, re-treatment marrow already undergoing a marrow procedure for non-research reasons, if a patie, if may be obtained in advance of planned treament, once a subject consent on 9296R.
For any bone marrow evaluation during the study period, we request the following specimens for research in addition to any marrow samples sent for clinical purposes:

- Approximately 7 ml of bone marrow aspirate (5 ml for patients 15-30 kg) in heparin (green top) tubes should be sent to FHCRC Research Cell Bank, Eastlake Building, E1-305 to be evaluated for the presence of adoptively transferred T cells by WT1 and/or EBV HLA-A*0201 peptide/multimer analysis and for other immunological parameters.

In patients with surgically accessible disease (disease that is accessible by needle or core biopsy, a malignant aspirate, or tumor that is surgically excised) in whom enough material can be obtained, single cells suspensions will be prepared to assess for T cell persistence and/or WT1 expression in the leukemia cells. All samples will be kept at room temperature and sent to the FHCRC Research Cell Bank, Eastlake Building, E1-305.

15.G.3. Assessment of Overall, T_{CM}, T_N and T_{EBV} Cell Persistence

Transferred TCR_{C4} CTL (overall persistence) will be enumerated by staining with TCR_{C4}-binding WT1 multimer in PBMC obtained at each time-points for as long as infused cells can be detected by multimer-binding (limit of detection approximately 0.03% of total $CD8^+$ T cells). DNA will also be isolated from blood and bone marrow samples and analyzed by PCR for WPRE as a marker of the presence of transduced T cells. HTTCS will then be used to characterize the presence and relative frequencies of individual T cell clonotypes derived from the T_N, T_{CM} and T_{EBV} subsets in the blood, bone marrow and/or extramedullary sites. HTTCS will be performed from samples drawn at day +7 (the peak of the response), at day +28 (establishment of short-term memory), at 3 months (long-term memory), and every 3 months thereafter while transduced cells are still detectable by multimer analysis. Clonotype frequencies >0.01% of $CD3^+$ T cells will be summated to determine relative frequencies of T_N-, T_{CM}- and T_{EBV}-derived at different time points. Because the sum of frequencies for each subset will likely still be >0.01% at 3-6 months, a dominant population (T_N, T_{CM} and T_{EBV}) will be defined as one in which the sum of its clonotype frequencies

reaches >80% of detectable transferred clonotypes. The time to disappearance of a transferred subpopulation will be defined as the time-point when the sum of transferred clonotype frequencies from that subset reaches 0.01% of $CD8^+$ T cells (see **Section 23.C**). Persistence of transferred TCR_{C4} WT1-specific T cells by multimer analysis and HTTCS will be performed in a similar fashion on specimens from any extramedullary sites, as they are available.

15.H. Efficacy Assessment (Exploratory Endpoint)

To assess the potential anti-leukemic effect of adoptively transferred T cells, bone marrow will be obtained to evaluate disease status by morphology, flow cytometry, and if appropriate by cytogenetics and molecular studies before and after treatment. For patients with a history of extramedullary disease, appropriate radiographic studies will be used to assess for treatment response or recurrence. Efficacy will be assessed by comparing outcomes of the study cohort with those in the non-treatment (observation) arm, as well as by determining treatment response in patients with measurable disease at the time of treatment.

15.H.1. For patients with measurable MRD: Patients with MRD detectable by flow cytometry at the time of study treatment will be evaluated at subsequent time points for eradication or reduction of MRD, as per response criteria described in **Appendix C**. Although patients will be enrolled to the study based on the presence of MRD by flow cytometry, some patients will also be expected to have MRD detectable by cytogenetics, FISH or PCR; therefore, appropriate patients will also be evaluated for evidence of eradiation or reduction in cytogenetic and molecular MRD, as per response criteria described in **Appendix C**.

15.H.2. The probability of relapse, disease-free survival and overall survival in the entire cohort of patients receiving the study intervention will be determined in comparison to those in the observation cohort (see **Section 23D**). The probability of relapse, disease-free survival and overall survival in both will also be determined in the subgroups of patients who are enrolled with MRD, as well as for the subgroup with CRi/CRp.

15.I. T_N, T_{CM} and T_{EBV} Migration to Tumor Sites (Exploratory Endpoint)

Localization of transferred TCR_{C4} T_N, T_{CM} and T_{EBV} cells to tumor sites will be evaluated in marrow samples obtained after infusions as well as in extra-medullary samples if available. DNA

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will be isolated from all samples to determine the presence and frequency of T_N, T_{CM} and T_{EBV} cells by HTTCS. To assess for the preferential localization of $T_N/T_{CM}/ T_{EBV}$, results will be compared to pre-treatment and concurrent PBMC values and, where possible, to pre-infusion extra-medullary tumor sample values. If enough material is obtained, single cell suspensions will be prepared and isolated by multimer staining to determine the phenotype and function of transferred CTL **(**see **Section 15.J)**

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

Persisting cells in blood, bone marrow and, if possible, tumor tissue, will be assessed for the surface expression of markers of central memory T cells (for example expression of CD28, CD27, CD127, CD62L and CCR7), markers associated with recent cell activation (for example CD69, CD25, CD107 and CD137), and markers associated with prolonged activation/exhaustion (for example PD1, TIM-3, LAG-3, CD160, 2B4, KLRG-1 and CD57). Results will be compared the original infusion products. The function of persisting TCR_{C4} cells will be assessed by measuring intracellular IFNγ, TNFα and IL-2 in response to stimulate with the WT1 epitope. Intra-nuclear Ki-67 expression will be used as an indication of *in vivo* proliferation. CFSE dilution will be used to assess *ex vivo* proliferative capacity in response to the WT1 epitope, and will be compared to the infusion product, as described[165,166]. HTTCS will be used to determine the origin and proportions of the persisting cells ($T_N/T_{CM}/ T_{EBV}$). The function, proliferation and proliferative capacity of T cells will be correlated with the type of persisting cells ($T_N/T_{CM}/ T_{EBV}$).

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

15.K.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), efforts will be made to obtain blood samples to test for replication competent lentivirus (RCL) at the following time points: Pretreatment, at 3, 6 and 12 months for the first year, and annually for up to 15 years. If all post-treatment assays are negative during the first year, subsequent yearly samples may be discontinued or may be obtained and be

archived for future analysis, and analyzed if clinical or scientific events make this indicated. Samples will be archived with appropriate safeguards to ensure long-term storage using a 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).

15.K.2 Long-Term Follow-Up

Efforts 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 new onset hematologic disorders. Additionally, samples will be collected to determine levels of gene-modified cells in peripheral blood for up to 15 years as recommended by FDA regulations. Suspect clinical symptoms or findings as noted above will trigger performing RCL analysis of archived samples and/or attempting to obtain additional samples, in consultation with CBER.

At clinic visits, patients will undergo physical examination and laboratory testing including CBC with differential, comprehensive chemistry 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 adverse events, including unexpected illnesses and hospitalizations. In general, most patients will be seen by their primary doctors in their local area, but the FHCRC in collaboration with Seattle Cancer Care Alliance (SCCA) will be available to

assist in the LTFU of participants in this clinical trial. If patients die or develop neoplasms, an effort will be made to assay for RCL and lentiviral integration in a biopsy sample of the neoplastic tissue or the pertinent autopsy tissue.

MANAGEMENT OF TOXICITIES AND COMPLICATIONS

16.A. Toxicity Grading

Toxicity grading will be evaluated according to the current guidelines in NCI CTCAE v4.0.[80] 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 (see **Section 16.E** and **Section 16.F**). If there is evidence of excessive unexpected treatment-related toxicity, the study stopping rules will apply (see **Section 23.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 **T cell infusions** and considered exceptions to criteria for discontinuation include:

i. Cytokine Release Syndrome (CRS) ≤ grade 3, 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, lasting <72 hours.
iv. Fever up to >40˚C that resolves within 48 hours of the T cell infusion.
v. Hypotension responsive to intravenous hydration and lasting for <72 hours, but not requiring pressor support.
vi. Grade 4 lymphopenia (lymphocytes < 200) that resolves within 3 weeks to baseline levels (pre-therapy).

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vii. 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 **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. Drop in blood pressure determined by physician to require 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.

16.B.3. Expected toxicities attributable to **cyclophosphamide and fludarabine conditioning** considered exceptions to criteria for discontinuation include:

i. Nausea, vomiting or diarrhea that resolves within 7 days.
ii. Lymphopenia (lymphocytes <200) that resolves within 3 weeks to baseline levels (pre-therapy).
iii. Thrombocytopenia (platelets <20,000) that resolves within 3 weeks to baseline levels (pre-therapy)
iv. Grade 4 neutropenia (neutrophils <500) that resolves within 3 weeks to baseline levels (pre-therapy).
    a. *Note that *febrile neutropenia* constitutes an expected toxicity in a proportion of patients treated with lymphodepleting chemotherapy. However, this will not be considered an exception to criteria for treatment discontinuation, as consideration to altering the lymphodepletion chemotherapy regimen will be made if stopping rules are met because of febrile neutropenia. For the purpose of this study, febrile neutropenia is defined as ANC<500/mm^3 with a temperature of 38.3^oC.

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

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16.C.1 Definition of Dose-Limiting Toxicities

Although unlikely based on prior experience, it is possible that the infusion of TCR_{C4}-transduced $CD8^+$ T cells will result in toxicities related to the recognition of normal tissues expressing WT1. It is anticipated that such symptoms and signs will occur within hours to 4 weeks after completion of the T cell infusion, though patients will continue to be followed for potential toxicities for 12 months after the last infusion, as described in **Section 15.E** and **Section 15.K.**

16.C.2 Non-hematologic toxicity requiring treatment discontinuation

This is defined as any grade 3 or 4 non-hematologic toxicity (CTCAE 4) that is deemed to be probably or definitely caused by infusion of the study treatment (i.e. not attributable to infection, recurrent/progressive AML, toxicity from prior therapies, or any identifiable cause other than conditioning chemotherapy, 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 four weeks after completion of the last T cell infusion although patients will be followed beyond four weeks for potential toxicities, as described in **Section 15.E** and **Section 15.K.** In patients who are still within the 12 month window of toxicity monitoring, but who no longer have any detectable WT1-specific T cells, new adverse events will not be attributed to WT1-specific T cells and will not be considered for treatment discontinuation.

16.C.3 Management of Non-hematologic toxicity

Any toxicities potentially caused by the T cell infusion and not attributable to a non-infusion related cause, occurring within 4 weeks of study treatment and requiring treatment discontinuation, will be managed by supportive care and steroids or tocilizumab at the discretion of the attending and the PI**.** This is outlined in **Section 16.E.** and **Section 16.F.**

16.C.4 Hematologic toxicity requiring treatment discontinuation

This is defined as any new or recurrent onset of thrombocytopenia (platelets < 20,000/mm^3) or otherwise grade 4 hematologic toxicity (blood/bone marrow CTCAE 4) that occurs at any time point after the first T cell infusion such as neutropenia (ANC < 500/mm^3) lasting > 2 weeks in patients not receiving chemotherapy and lasting ≥ 3 weeks in patients receiving chemotherapy, in the context of new bone marrow aplasia (< 5% cellularity), and which is attributed to the study treatment (i.e. not associated with prior chemotherapy, tumor progression in the bone marrow, infection, medications, or any identifiable cause other than T cell infusion or IL-2 administration).

In patients with incomplete count recovery (CRi/CRp) prior to the study intervention, we will consider grade 4 hematologic toxicity requiring treatment discontinuation as a sustained drop in ANC below 20% of the starting measurement and/or in platelets below 20% of the starting measurement lasting > 3 weeks and which is attributed to the study treatment. In patients who are still within the 12 month window of toxicity monitoring, but who no longer have any detectable WT1-specific T cells, new adverse events will not be attributed to WT1-specific T cells and will not be considered for treatment discontinuation.

16.C.5 Management of hematologic toxicity

If a patient develops new onset hematologic toxicity lasting at least 2 weeks in patients not receiving chemotherapy and lasting ≥ 3 weeks in patients receiving chemotherapy, defined herein as ANC <500/mm^3 or platelets <20,000/mm^3 in a patient with pretreatment laboratory values of ANC >1000/mm^3 and platelets >100,000/mm^3, and which cannot be explained by alternative cause such as known disease progression, infection, or other medications, a bone marrow sample will be obtained. As mentioned above, in patients with incomplete count recovery (CRi/CRp) prior to the study intervention, we will consider grade 4 hematologic toxicity requiring treatment discontinuation as a sustained drop lasting at least one week in ANC below 20% of the starting measurement and/or in platelets below 20% of the starting measurement.

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 does not reveal aplasia or disease progression, and if no other clear etiology for marrow suppression is found, patients will be observed for seven more days. If by that time there is no improvement of bone marrow function and no clear etiology for marrow suppression is still 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 seventh 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.

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

16.D. Management of Symptoms During T cell Infusions

Immediate reactions to infusions (i.e. 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.

16.D.1. Milder reactions (i.e. <grade 3 CTCAE v.4 or less severe than specified below) Including 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) ≤150 or ≤50 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.

Suggested Management may include decreasing the rate of infusion and/or 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.

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16.D.2. More severe reactions

Including symptoms such as:

- Hypotension with systolic BP <90 mmHg and >20 mmHg below baseline.
- Tachycardia with HR >150 and >50 above baseline.
- Tachypnea with RR >32 and >10 above baseline.
- Hypoxemia with O_2 saturation of <88% and >5% fall from baseline.

Suggested Management may include modifying the infusion rate or 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 may 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 after discussion with the PI or designee 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, and on specified time points after the T cell infusion as indicated in **Appendix B.** 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 IFNγ, TNFα, 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 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 and/or progressive symptoms and signs may result in the administration of corticosteroids and/or tocilizumab as described in **Section 16.F.**

16.F. Management of Severe Treatment-related Toxicities

1. If a new and unexpected CTCAE v4.0 grade ≥3 toxicity is observed following T cell infusion, the patient will receive medical treatment appropriate for the clinical abnormality.
2. A new and unexpected grade ≥3 toxicity that is attributable to the T cell infusion, is unresponsive to supportive measures, or persists >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), and/or tocilizumab (anti-interleukin-6 receptor) after discussion with the PI. *These are general guidelines for the glucocorticoid administration and taper and may 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 0.5-1mg/kg/day p.o.**
Day 5-12	**Taper p.o prednisone by 20% q 2 days**

3. Patients who require corticosteroids for treatment of severe toxicities will not receive additional T cell or IL-2 treatment on this protocol and will continue to be followed for toxicity until symptoms have resolved. Methylprednisolone (or an equivalent dose of an alternative corticosteroid) can be tapered more rapidly when symptoms are significantly improving or WT1-specific T cells are <0.05% of CD8+ cells in peripheral blood.

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4. It is suggested that patients receiving corticosteroids have daily assessments of peripheral blood for the presence of WT1-specific CTL cells (vβ17+ and multimer+) for 5 days and then at least weekly for four weeks. 20 mL of blood (or up to 1 mL/kg in patients 15-20 kg) 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 per clinical judgment.

16.G.2 Patients with extramedullary disease may receive radiation therapy at the discretion of their treating physician and consulting radiation oncologist.

16.H. Off-study Criteria

A patient's participation on the protocol will be terminated for any of the following reasons and the reasons for premature discontinuation must be recorded on the case report form.

- If the sponsor decides to stop the study
- The participant withdraws consent.
- Patient death.
- At the discretion of the PI

MANAGEMENT OF T CELL INFUSIONS IN PATIENTS WHO RELAPSE OR PROGRESS DURING T CELL THERAPY

Patients who started treatment with no evidence of disease and relapsed during T cell therapy, or patients who started treatment with evidence of disease and experience progression of their disease during T cell therapy will be evaluated for the characteristics of the leukemic cells and advised about treatment options by their treating physician.

T cell therapy may be continued if relapse or disease progression occurs prior to the last dose of T cells infusions, or at the discretion of the PI. The T cell infusions may be interrupted to allow administration of cytoreductive therapy. Patients who receive cytoreductive chemotherapy may resume treatment with WT1-specific T cells after evidence of recovery of hematopoiesis (ANC >200/mm^3 and plts >20,000/ mm^3).

This decision to continue WT1-specific T cell therapy despite disease progression is based on the extremely poor prognosis for these patients, the fact that they might benefit from infusion of available WT1-specific T cells, and the possibility of acquiring insights into safety and potential anti-leukemic effects of WT1-specific T cells.

Patients whose T cell therapy is interrupted for cytoreductive therapy will not undergo protocol specific evaluations, AE assessments or sample collections until T cell therapy resumes.

OPTIONS FOR FURTHER TREATMENT AFTER COMPLETION OF STUDY THERAPY

If adoptively transferred WT1-specific T cell infusion demonstrated anti-leukemic activity in the patient, the option of additional T cell infusions may be discussed with the PI, the IND Sponsor, the patient and the treating physician.

TARGETED/PLANNED ENROLLMENT

This study plans to treat 35 individuals with AML over 3 years.

Table 5. Ethnic and gender distribution chart

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TARGETED/PLANNED TREATMENT: Subjects					
Racial Categories	Ethnic Categories				
	Not Hispanic or Latin		Hispanic or Latino		Total
	Female	Male	Female	Male	
American Indian/Alaska Native	1	0	0	0	1
Asian	2	2	0	0	4
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	0	1	0	0	1
White	13	14	1	0	28
More Than One Race	0	0	0	1	1
Racial Categories: Total of All Subjects	16	17	1	1	35

These targeted/planned enrollment numbers are based on relative percentages of race/ethnicity reported for newly diagnosed AML patients treated at the SCCA/FHCRC in 2013-2014, and are reflective of the frequencies in our catchment area.

GUIDELINES FOR ADVERSE EVENTS REPORTING

20.A. Reporting of Adverse Events (AEs)

All unexpected and serious adverse events attributed 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 T cell infusion through 4 weeks after the last T cell infusion. Beginning 4 weeks after the last T cell infusion, only study-related SAEs may be recorded. For those patients with an interval of > 1 month between T cell infusions, collection of all grade ≥ 3 AEs will be temporarily put on hold, and then will resume at the onset of any additional study treatments.

20.B. Reporting to the FDA

As a study conducted under IND (Investigational New Drug) regulations we will comply with the FDA regulations regarding safety reporting 21CFR312.32 including the following requirements:

1. A sponsor must promptly review all information relevant to the safety of the drug

21CFR312.32 (b).

2. A sponsor must notify FDA in an IND safety report of potential serious risks, as soon as possible but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting under 21 CFR312.32 (c)(1). Information that is required to be reported includes, but is not limited to, a. Serious and unexpected adverse reactions and b. An increased rate of occurrence of serious suspected adverse reactions.

3. The IND safety report must be completed and sent to the FDA in a narrative format, on FDA Form 3500A, or an electronic format.

4. A sponsor must also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information 21CFR312.32 (c)(2).

20.C. Definitions

Definitions associated with reportable events can be found on the FHCRC IRO extranet website. (Relevant FHCRC policies include, but are not limited to the documents listed in **Table 6**. Please also refer to the FHCRC IRO website.)

Table 6. 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

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DATA AND SAFETY MONITORING PLAN

21.A. Primary Monitoring

The PI of the study and the IND Sponsor will have primary responsibility for ensuring that the protocol is conducted as approved by the Scientific Review Committee and IRB. The PI and the IND Sponsor 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 adverse events are reported according to protocol guidelines, and that any adverse reactions reflecting patient safety concerns are appropriately reported.

21.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 his/her 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.

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

The study will be monitored by the Immunotherapy Integrated Research Center (IIRC) DSMB. 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 the study Sponsor 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 DSMB is an independent, multidisciplinary group consisting of clinical experts and a statistician who collectively have experience in leukemia, lymphoma, hematology, biostatistics, and the conduct and monitoring of clinical trials. The DSMB will meet approximately every 6 months to review data. The current members are listed in the IIRC DSMB charter.

Institutional support of trial monitoring will be in accordance with the FHCRC/University of Washington Cancer Consortium Institutional Data and Safety Monitoring Plan. Under the provisions of this plan, FHCRC Clinical Research Support coordinates data and compliance monitoring conducted by consultants, contract research organizations, or FHCRC employees unaffiliated with the conduct of the study. Independent monitoring visits occur at specified intervals determined by the assessed risk level of the study and the findings of previous visits per the institutional DSMP.

In addition, protocols are reviewed at least annually and as needed by the Consortium Data and Safety Monitoring Committee (DSMC), the FHCRC Scientific Review Committee (SRC), and the FHCRC/University of Washington Cancer Consortium Institutional Review Board (IRB). The review committees evaluate accrual, adverse events, stopping rules, and adherence to the applicable data and safety monitoring plan for studies actively enrolling or treating patients. The IRB reviews the study progress and safety information to assess continued acceptability of the risk-benefit ratio for human subjects. Approval of committees as applicable is necessary to continue the study.

The trial will comply with the standard guidelines set forth by these regulatory committees and other institutional, state and federal guidelines.

21.C.3 Statistical Monitoring Guidelines

The DSMB will review all grade III or greater toxicities as defined by version 4.0 of NCI CTCAE, and as further described in **Section 16** of this protocol and determine whether the study should be prematurely discontinued due to toxicity. The clinical investigators assessing patients will be responsible for evaluations to grade toxicity. Criteria for discontinuing therapy in an individual patient are described in protocol section titled “Management of Toxicities and Complications”. Criteria and procedures for discontinuing the trial are described in the sections titled “Data and Safety Monitoring” and “Statistical Considerations”.

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 will be summarized in a table. Tumor responses will be determined as specified above.

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 and/or data coordinator will maintain a Case Report Form (CRF) for each patient treated on this protocol. The CRFs 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 and/or secured computers. 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.

STATISTICAL CONSIDERATIONS

23.A. Analysis of Safety/Toxicity (Primary Endpoint)

The primary objective of this trial is to examine the safety of adoptive T cell therapy using autologous $CD8^+$ T cells genetically modified to express a high affinity WT1-specific TCR for patients with high-risk AML. There is already an extremely high likelihood of toxicity with

chemotherapy in this high-risk leukemic population. In published trials of patients receiving cytarabine-based consolidation therapy, up to 57% of enrolled patients have been shown to develop grade 3 or 4 non-hematologic organ (e.g. lung, liver, kidney) toxicities and/or infectious complications. [167-169] Considering the toxicity associated with undergoing re-induction chemotherapy for relapse, roughly 70% of older patients, based on published toxicity data from induction chemotherapy regimens in this population, would be expected to develop grade 3 or 4 non-hematologic organ toxicity and/or infectious complications.[83] It is expected that nearly all patients receiving induction or consolidation chemotherapy will exhibit grade 3 or 4 hematologic toxicity following treatment.

Given this extremely high degree of expected toxicity with chemotherapy, and in light of the overall very poor prognosis of patients with disease relapse, we will consider treatment with WT1-specific T cells to have an acceptable safety profile if the true rate of grade 3 or 4 non-hematologic toxicity (as defined in **Section 16.C.2**) and grade 4 hematologic toxicity (as defined in **Section 16.C.4**) following WT1-specific T cells is less than 50%. Therefore, if there is sufficient evidence to suggest that the true toxicity rate exceeds 50%, the study will be suspended pending review by the DSMB. Sufficient evidence will be taken to mean any observed proportion of toxicities for which the associated one-sided lower 90% confidence limit exceeds 50%.

23.A.1. Safety analysis for sequential cohorts in Stage 1

A total of 5 patients are planned to be treated, sequentially, in each cohort. The cohort size of 5 patients is based on feasibility rather than statistical considerations. Before allowing enrollment to a subsequent cohort, a full assessment of safety and other elements (such as T cell persistence) will be performed. The study team will make this assessment. Because of the length of time required to generate transduced T cells, we will continue to screen and enroll, and generate T cell product at the time of inter-cohort safety analysis, but will not begin treatment on the next cohort prior to reviewing safety data.

If toxicity as defined above develops in 3 of 5 patients at any cohort specified, the study will be put on hold until such time as the DSMB can review the toxicity data. If 2 or fewer patients develop dose-limiting toxicity, as defined above, the next group of 5 patients will be treated on the next cohort, according to the respective treatment plan, if deemed suitable by the study team.

23.A.2. Safety, persistence and efficacy analysis for determination of treatment regimen used in Stage 2

At the completion of stage 1, the study team and DSMB will convene for a review of available toxicity data, as well as a review of T cell persistence data and any available efficacy data as noted above. Completion of stage 1 will be defined as the treatment and toxicity assessment (defined as administration of at least one T cell infusion, followed by safety monitoring for >28 days) for 15 patients across 3 cohorts.

Comparative toxicities, both expected and unexpected, as detailed in **Sections 16.B and 16.C** will be reviewed for all 3 cohorts. In terms of comparing percentage of $CD8^+$ T cells at 30 days, if the true mean percentage in two groups is separated by 1.5 standard-deviation units, 5 patients per group will yield 83% power to observe a statistically significant difference in percentage of persisting infused T cells present at 30 days (at the one-sided significance level of 0.10). We will not, however, require a statistically significant difference in persistence to take one dose forward, rather we shall take forward the dose that yields the highest average percentage of persisting infused $CD8^+$ cells at day 30, provided this dose has an acceptable safety profile. The study team and the DSMB will meet to review these data and will make a recommendation on which dose to move forward to the second stage of the trial.

The group will make an informed decision as to which treatment plan should be advanced for Stage 2 testing (which T cell components will be used and if lymphodepletion chemotherapy will be modified or discontinued.) It is planned to treat 20 additional patients in stage 2 with the selected plan.

23.A.3. Safety analysis in Stage 2

A total of 20 patients are planned to be treated in stage 2. We will include the initial 5 patients from the selected cohort in the overall toxicity analysis for a total of 25 patients considered for the respective therapy. If there is sufficient evidence to suggest that the true toxicity rate exceeds 50%, the study will be suspended pending review by the DSMB. Sufficient evidence will be taken to mean an observed proportion of toxicities for which the associated one-sided lower 90% confidence limit exceeds 50%, which will be determined after every 5^{th} patient becomes

evaluable. Operationally, any of the following observed ratios of toxicities/treated patients would yield such a confidence limit and would warrant suspension of the study and DSMB review: 4/5, 7/10, 10/15, or 13/20. If the true probability of toxicity is 40%, then the probability of reaching one of the above ratios after 20 patients is approximately 0.12. For a true probability of toxicity of 70%, the probabilities of one of these ratios occurring is approximately 0.86 (estimated from 5,000 simulations).

23.B. Analysis of the Feasibility of infusing TCR_{C4} Transduced T Cells (Primary Endpoint)

Feasibility is defined as the ability to reproducibly generate and infuse the T cells for eligible patients. We anticipate a 4-6 week period required for T cell generation. We will enroll patients until we are able to isolate, grow, and infuse T cells to at least 35 patients, presuming that the trial has not been suspended due to safety reasons. The proportion of patients for which this is possible will be estimated, and our goal is that at least 80% of patients will be treated, excluding patients who could not receive products because of frank relapse, uncontrolled infections or other patient-related factors. No formal statistical considerations will be used to evaluate this endpoint beyond a simple estimate of the proportion along with its 95% confidence interval.

23.C. Analysis of persistence in blood or bone marrow of TCR_{C4}-transduced cells
Transferred TCR_{C4} CTL will be enumerated in real time by staining with TCR_{C4}-binding WT1 multimer in PBMC obtained from patients prior to T cell infusions (baseline) and on days 1, 7, 14, 21 and 28, at 2 months and 3 months, and then every 3 months during the interval between infusions. Following the second infusion, PBMC will be analyzed for persistence on days 1, 7, 14, 21 and 28, monthly x 2, and then every 3 months until WT1-specific T cells are no longer detectable by analysis (limit of detection 0.03% of total $CD8^+$ T cells). Persistence will be measured and presented descriptively. Migration of T cells to bone marrow will be compared to that that in peripheral blood using a paired t-test. If the true distribution of differences (bone marrow – peripheral blood) is 0.5 standard-deviation units from zero, 25 patients will provide 87% power to observe a statistically significant difference between bone marrow and peripheral blood. HTTCS (limit of detection 0.01% of $CD3^+$ T cells) will be used to distinguish the frequency of individual T cell clonotypes from the T_N, $T_{CM, or}$ T_{EBV} subsets at day 7 (the peak of the response), at day +28 (establishment of short-term memory), at 3 months (long-term memory), and every 3 months thereafter (at time points blood or marrow samples are obtained) as long as persistence

by multimer is observed. Clonotype frequencies >0.01% of $CD3^+$ T cells will be summated to determine relative frequencies of T_N and T_{CM} at different time points. Because the sum of frequencies for each subset will likely still be >0.01% at 3-6 months, the population persisting less well will be identified when the sum of its clonotype frequencies reaches <25% of detectable transferred clonotypes. At each time point, we will assess the proportion of T cell subsets and test the null hypothesis that the proportions are equal at each time. We'll compare each subset to a fixed hypothesized frequency of 33%. With 25 patients, a true effect size (frequency of cells divided by standard deviation of the frequency) of 0.5 standard-deviation units leads to 87% power to be able to conclude that the proportion of a specific T cell subset is statistically significantly different (at the one-sided significance level of .10) from 33%.

23.D. Assessment of efficacy (Exploratory Endpoint)

We shall also summarize the probability of relapse/progression using cumulative incidence estimates, where death without relapse/progression will be regarded as a competing risk. In addition, Kaplan-Meier estimates will be used to estimate overall and disease-free survival. Efficacy assessment will be performed without distinction between T_N vs T_{CM} vs T_{EBV}, as this trial is not designed to differentiate between relative efficacy of these three populations, but rather to assess T cell therapy targeting WT1 overall, and to gain insights into the subpopulation of cells that appears to provide the most functional and persistent response.

In order to gain a preliminary impression of potential efficacy, we will also follow patients who signed the study consent, but did not receive treatment, because they were not HLA-A*02:01, , they did not meet treatment criteria, or due to their preference (anticipated "observation" cohort size is 35). The populations being treated are at high risk of relapse. Patients with adverse-risk disease, MRD or poor count recovery after induction or with relapsed disease have a 1-year relapse-free survival of ~10-25% without HCT, [21-23,47] suggesting impact on relapse should be evident within 1-2 years of T cell infusions. Although the non-randomized nature of treated and untreated groups precludes definitive efficacy conclusions, this cohort of non-HCT patients (who will be receiving current standard or care) is considered a better benchmark by which potential efficacy can be assessed than an historical control group. Keeping in mind these limitations, if true 1-year relapse rates are 50% (treated) and 75% (control), 25 treated and 35 control patients,

respectively, provide 83% power to observe a statistically significant difference (one-sided significance level of .10).

We will also consider clearance of MRD in determining efficacy of the T cells. We will estimate the percent of MRD positivity at 6 months for patients who were treated with MRD, which is defined as any detectable blast count by flow cytometry, or by persistently abnormal cytogenetics/FISH or PCR testing in patients with relevant disease markers. Corresponding confidence intervals will accompany each of these estimates. While sample size is not contingent upon a specific benchmark for elimination of MRD, 20% of MRD^+ treated patients who have no detectable MRD at 6 months would be considered a sufficiently encouraging result to warrant further study.

ADMINISTRATIVE CONSIDERATIONS

24.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.

24.B. Termination of Study

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

- All 35 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 and the IND Sponsor reserves the right to terminate the study at any time. The FDA may also terminate the study.

APPENDICES

APPENDIX A

Karnofsky and Lansksy Scales			
	Karnofsky Scale (age ≥ 16 years)		**Lansky Scale (age <16 years)**
	Able to carry on normal activity; no special care is needed.		**Able to carry on normal activity; no special care is needed.**
100	Normal, no complaints, no evidence of disease	**100**	Fully active
90	Able to carry on normal activity	**90**	Minor restriction in physically strenuous play
80	Normal activity with effort	**80**	Restricted in physically strenuous play, tires more easily, otherwise active
	Unable to work, able to live at home and care for most personal needs; a varying amount of assistance is needed		**Mild to moderate restriction**
70	Cares for self, unable to carry on normal activity or to do active work	**70**	Both greater restrictions of and less time spent in active play
60	Requires occasional assistance but is able to care for most needs	**60**	Ambulatory up to 50% of time, limited active play with assistance/supervision
50	Requires considerable assistance and frequent medical care	**50**	Considerable assistance required for any active play; full able to engage in quiet play
	Unable to care for self, requires equivalent of institutional or hospital care, disease may be progressing rapidly		**Moderate to severe restriction**
40	Disabled, requires special care and assistance	**40**	Able to initiate quiet activities
30	Severely disabled, hospitalization indicated, although death not imminent	**30**	Needs considerable assistance for quiet activity
20	Very sick, hospitalization necessary	**20**	Limited to very passive activity initiated by others (e.g., TV)
10	Moribund, fatal process progressing rapidly	**10**	Completely disabled, not even passive play

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APPENDIX B

Monitoring Schedule*testing

Event		History and PE	Chemistry Panel	CBC	UA	Blood Draw for research[&,#]	Other^	Bone Marrow Evaluation[@]
Enrollment		X	X	X			X	
Leukapheresis		X	X	X				
Pre-treatment		X	X	X	X	X	X	X (within 6 weeks of first infusion)
Infusion #1	Day 0	X	X	X		X		
	Day +1-+4	X	X	X		X		
	Day +7, +14, +21	X	X	X	X (D14)	X		
	Day +28	X	X	X	X	X		X (only if delaying 2nd infusion ≥ 4 weeks)
After first infusion	Monthly for 2 additional months then q 3 months	X	X	X		X		
Pre-infusion #2					X			X (only if ≥ 10 weeks elapsed since first infusion)
Infusion #2	Day 0	X	X	X		X		
	Day +1-+4	X	X	X		X		
	Day +7, +14, +21	X	X	X	X (D14)	X		
	Day +28	X	X	X	X	X		X (4 weeks after infusion)
At completion of study treatment%	At 2, 3, 6 and 12 months	X	X	X		X		
12 months to 15 years%$		X[$]				X[$]		

* The dates listed on the study calendar above are approximate as patients may not always be able to follow the time points as dictated by the protocol.
[&] Peripheral blood in green-top (heparin) tubes: 60 ml for adults and children > 50kg; 30 ml for children 15-30kg; and 1ml/kg in children 15-30kg.
[#] Peripheral blood in serum separator (gold top) tubes: 10ml will be collected for adults and children > 50kg; 5 ml for children 15-30kg; and 3 ml in children 15-30kg.
[@] Bone marrow specimens to be collected: 7 ml in heparin for adults and children > 30kg; 5 ml for children 15-30kg;
%: The following time points are recommendations for patient follow-up and may be completed by local providers.
^: "Other" testing includes determination of WT1 expression of leukemic blasts in blood or marrow, and HLA-A*0201 expression in patient cells from blood at the time of screening. Additionally, this includes HIV viral load testing, both at the time of enrollment, and also within 3 months of treatment, as needed.
[$] Long Term Follow Up monitoring will begin at 12 months following the 2nd T cell infusion. During this period, patients should be monitored clinically as per FDA guidelines for up to 15 years. Research blood draws may be requested to detect persistence of WT1-specific T cells up to every 3 months until multimers are <0.03% CD8+ cells.

APPENDIX C

AML Response Criteria[a,b]

Criteria	**Complete Response (CR)**	**Partial Response (PR)**	**Stable Disease (SD)**
Morphologic	Bone Marrow blasts <5%; no blasts with Auer rods; no extramedullary disease Transfusion independent Platelets ≥ 100,000/μl Absolute neutrophil count >1000/μl (CRi: incomplete hematologic recovery CRp: incomplete platelet recovery)	Decrease of at least 50% in the percentage of blasts to 5-25% in the bone marrow and the normalization of blood counts as for CR. (PRi and PRp if incomplete hematologic or platelet recovery)	Failure to achieve at least PR, but no evidence of progression for >4 weeks.
Cytogenetic	Disappearance of previous cytogenetic abnormality	≥ 50% reduction of abnormal metaphases	See above
FISH	Disappearance of previous FISH abnormality	As defined by individual assay.	See above
Molecular	Disappearance of molecular mutation/marker	As defined by individual assay.	See above
Flow cytometric	Disappearance of cells with aberrant phenotype.	≥ 10 fold reduction in percentage of leukemic cells if initial percentage of BM blasts is ≤ 5%. Otherwise follow morphologic criteria.	See above

[a] Cheson BD, Bennett JM, Kopecky KJ, et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol. Dec 15 2003;21(24):4642-4649.
[b] Cheson BD, Greenberg PL, Bennett JM, et al. Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. Blood. Jul 15 2006;108(2):419-425.

APPENDIX D

Disease risk stratification based on leukemia-associated cytogenetic and molecular abnormalities, as per 2017 European LeukemiaNet (ELN) guidelines

Risk Category[b]	Genetic Abnormality
Favorable	t(8;21)(q22;q22.1); ***RUNX1-RUNX1T1*** inv(16)(p13.1q22) or t(16;16)(p13.1;q22); ***CBFB-MYH11*** Mutated ***NPM1*** without ***FLT3***-ITD or with ***FLT3***-ITD$^{low(c)}$ Biallelic mutated ***CEBPA***
Intermediate	Mutated ***NPM1*** and ***FLT3***-ITD$^{high(c)}$ Wild type ***NPM1*** without ***FLT3***-ITD or with ***FLT3***-ITD$^{low(c)}$ (w/o adverse-risk genetic lesions) t(9;11)(p21.3;q23.3); ***MLLT3-KMT2A***[d] Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); ***DEK-NUP214*** t(v;11q23.3); ***KMT2A*** rearranged t(9;22)(q34.1;q11.2); ***BCR-ABL1*** inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); ***GATA2,MECOM(EVI1)*** -5 or del(5q); -7; -17/abn(17p) Complex karyotype,[e] monosomal karyotype[f] Wild type ***NPM1*** and ***FLT3***-ITD$^{high(c)}$ Mutated ***RUNX1***[g] Mutated ***ASXL1***[g] Mutated ***TP53***[h]

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