Administration of HIV-specific T cells to HIV+ Patients Receiving High Dose Chemotherapy Followed by Autologous Stem Cell Rescue -Auto-RESIST

BMT CTN PROTOCOL 1903 (AMC-109)
VERSION 4.0

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National Heart, Lung, and Blood Institute
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PROTOCOL SYNOPSIS - BMT CTN PROTOCOL #1903

Administration of HIV-specific T cells to HIV+ Patients Receiving High Dose Chemotherapy Followed by Autologous Stem Cell Rescue - Auto-RESIST

Study Chairpersons: Dr. Richard Ambinder and Dr. Kieron Dunleavy

Protocol Officer: Dr. Steven Devine

Primary Objective: The primary objective is to determine 1.) the proportion of participants who can be treated with **HIV antigen-specific T-cells Targeting Conserved Epitopes** (HST-NEETs) within 1 week of autologous hematopoietic stem cell transplantation (ASCT) in a cooperative multi-institutional setting and 2.) the efficacy of HST-NEETs in reducing the HIV intact proviral reservoir at 6 months after ASCT.

Secondary Objectives: Participants will be assessed for the following endpoints:

1. Progression-free survival at 6 months and 1-year post-ASCT;
2. The incidence and severity of acute infusion related toxicities;
3. Impact of therapy on the HIV intact proviral reservoir at 1-year post-ASCT.

Exploratory Objectives: Participants will be assessed for the following:

1. CR and CR+PR rates at Day 100 post-ASCT;
2. Overall survival at 6 months and 1-year post-ASCT;
3. Time to hematopoietic recovery;
4. Incidence of infections at 1-year post-ASCT;
5. Non-relapse mortality at 6 months and 1-year post-ASCT;
6. Toxicities through 1-year post-ASCT;
7. Assessment of plasma DNA in blood (clonal Ig DNA) as a tumor marker at Day 100, 6 months and 1-year post-ASCT;
8. Impact of therapy on the HIV intact proviral reservoir at Day 100 post-ASCT;
9. HIV RNA in plasma at Day 100, 6 months, and 1-year post-ASCT;
10. Impact of therapy on the total proviral HIV DNA at Day 100, 6 months, and 1-year post-ASCT.
11. HST-NEETs persistence and expansion *in vivo.*

Study Design: This is a Phase II multi-center trial single arm trial of autologous transplantation (ASCT) followed by administration of HST-NEETs for treatment of HIV associated lymphoma.

Accrual Objective: The trial will enroll 12 participants.

Accrual Period: The estimated accrual period is four years.

Eligibility Criteria: Eligible participants are HIV positive and plan to be treated by high dose chemotherapy followed by an autologous stem cell transplant (ASCT). Participants are a minimum of 15 years of age with Karnofsky performance status greater than or equal to 70% that have primary diffuse large B-cell, immunoblastic, plasmablastic, high grade, Burkitt, primary effusion lymphoma, or classical Hodgkin lymphoma. -Participants must have chemosensitive disease as demonstrated by complete or partial response to induction or most recent salvage chemotherapy. Participants cannot have had prior autologous, allogeneic HCT or CAR T-cell therapy. Participants must initiate conditioning therapy within 3 months of stem cell mobilization or bone marrow harvest. Blood cell mobilization or bone marrow harvest will be carried out per institutional guidelines. Participants may not have HIV refractory to pharmacologic therapy. Patients must not have an uncontrolled infection. Participants must not have received previous cellular therapy.

Treatment Description: Participants will either have 100-120 mL of peripheral blood drawn or 80-100 mL of mononuclear cells (MNCs) collected through apheresis and sent to Children's National Hospital for manufacturing of HST-NEETs 6 weeks prior to ASCT. Participants will receive Carmustine (BCNU) 300 mg/m^2 Day -6, Etoposide (VP-16) 100 mg/m^2 BID Days -5 to -2, Cytarabine (Ara-C) 100 mg/m^2 BID Days -5 to -2, and Melphalan 140 mg/m^2 Day -1 followed by ASCT on Day 0 and will receive one dose of HST-NEETs (2 x10^7 cells) between Days +3 to +7.

Study Duration: Participants will be followed on study for one-year post-ASCT.

Interim Analysis: No interim analyses for efficacy or futility are planned.

Stopping Guidelines: Two key safety endpoints (Day 30 treatment related mortality and Grade 3 or higher infusion-related toxicities lasting greater than 24 hours) will be monitored. Any HST-NEET treatment related mortality within 30 days of transplantation will trigger a safety review prior to treatment of subsequent study participants. Three or more infusion events will trigger consultation with the Data and

Safety Monitoring Board (DSMB). In the event of graft failure following HST-NEETs infusion, the protocol will be temporarily halted and reviewed by the NHLBI DSMB. Graft failure will be defined as a failure to achieve three consecutive labs of greater than or equal to 500 neutrophils/µL by Day 100**.**

Correlative Studies: Blood will be collected for monitoring the persistence/expansion of the infused T cells by ELIspot, multimer analysis, intracellular cytokine staining and TCR sequencing. We will also assess intact proviral DNA in peripheral blood mononuclear cells as a measure of the HIV reservoir, and clonal immunoglobulin DNA in plasma as an indicator of minimal residual disease/early relapse.

STUDY SCHEMA

6 Weeks Pre-ASCT	• 100-120mL Blood Drawn or apheresis collection[5] for HST-NEETs Manufacturing[1] • 70 mL Blood Drawn for Intact Proviral DNA Assay (IPDA)[2] • 5 mL Blood Drawn for Single Copy HIV-1 RNA Assay[3] Disease Assessment
Day -6 to -1	BEAM Conditioning Regimen
Day 0	Autologous Stem Cell Transplant
Day +3 to Day +7	HST-NEETs (2x 10^7 cells/m^2) Administered[4]
Day 100 Post-ASCT	• 70 mL Blood Drawn for IPDA** • 5 mL Blood Drawn for Single Copy HIV-1 RNA Assay[3] • Disease Assessment
6 Months Post-ASCT	• 70 mL Blood Drawn for IPDA** • 5 mL Blood Drawn for Single Copy HIV-1 RNA Assay[3] • Disease Assessment
12 Months Post-ASCT	• 70 mL Blood Drawn for IPDA** • 5 mL Blood Drawn for Single Copy HIV-1 RNA Assay[3] • Disease Assessment

[1]Collection should be scheduled with Children's National Hospital at least 2 weeks prior to acquisition. Sample should be shipped at ambient temperature overnight to Children's National Hospital. The standard manufacturing process takes 3 to 6 weeks.

[2] Sample should be shipped to Children's National Hospital.

[3] Sample should be shipped to University of Pittsburgh School of Medicine.

[4]HST-NEETs will be shipped to site. Study staff will thaw and administer cells. If the ideal window of 3-7 days post-transplant is missed, the cells may still be administered up to day 30 and the participant will continue study follow-up.

[5] The participant will have their cells collected via apheresis instead of a standard blood draw if the ALC is <800/uL but above 400/uL; see Section 2.4.1 for additional details.

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CHAPTER 1

1. BACKGROUND AND RATIONALE

1.1. ASCT in Patients with HIV Infection

The Blood and Marrow Clinical Trials Network (BMT CTN) has previously demonstrated that autologous stem cell transplant (ASCT) is a safe and potentially curative therapy for human immunodeficiency virus (HIV)-associated lymphomas in people living with HIV.[1] For some patients, allogeneic transplantation is the preferred option and anecdotally has led to the eradication of the HIV-reservoir. More than ten years ago, Timothy Ray Brown ('the Berlin Patient') received an alloBMT for treatment of acute myeloid leukemia (AML) from a donor who was homozygous for the CCR5Δ32 variant and was cured.[2] Recently, it was reported that a patient with Hodgkin lymphoma ('the London Patient') achieved long-term HIV-1 remission following alloBMT with a donor homozygous for CCR5Δ32.[3] The 32-base pair deleted variant of CCR5 affects about 1% of Caucasians and results in a non-functional CCR5 receptor with resistance to infection with the most common strains of HIV (R5).[4] CCR5Δ32/Δ32 donors are relatively rare. Moreover, for many patients with HIV and refractory/relapsed lymphoma, an ASCT would be the recommended treatment over an allogeneic transplant, and thus, using a CCR5Δ32/Δ32 donor would not be an option. Recent data suggest it may be possible to impact the long-term HIV reservoir using autologous HIV-specific T cells in the immediate post-ASCT setting. This is the focus of the present study.

As noted above, most HIV-infected individuals do not have CCR5Δ32/Δ32 donor options. Instead, donors who are not HIV-resistant are used. A recent report indicated that in these patients, reconstitution of the T cell compartment after allo-HSCT was slow and heterogeneous and that HIV-specific T cells had limited functionality and that an initial short phase of high T cell activation may constitute a window of vulnerability for the reseeding of viral reservoirs[5]—a finding that suggests there may be a role for adoptive T cell therapy in alloBMT for HIV patients as well.

1.2. T Cell Therapy in HIV-Infected Individuals

1.2.1. Previous Experience Using HIV-Specific T-Cells

The feasibility and safety of infusing HIV-infected subjects with ex vivo-expanded autologous HIV-specific T-cells was demonstrated in several studies.[6,7,8,9] The earliest study of ex vivo expanded HIV-specific T cells in actively viremic patients found that infusion with up to 10^{10} non-specifically activated, expanded autologous CD8+ T cells was safe and tolerable with no cytotoxic T-lymphocyte (CTL) related adverse events in 5 patients with AIDS, but enhancement of HIV specific cytotoxicity post infusion was transient and no impact on virologic outcome was found.[10] In another pilot study of actively viremic participants with Chronic HIV Infection (CHI), autologous administration of CD8+ CTLs primed to express cytotoxicity against HIV-1 epitopes gp120, gag p17, p24m and nef produced no toxicity or clinical deterioration over six months of follow-up.[6] Three of six participants showed improvements in plasma HIV RNA levels or CD4

count at six months despite low enrollment CD4 counts (100-400 /mm3). Two additional studies of one patient each who received an infusion of CTL clones against Gag and Nef, respectively, did not find any impact on viremia due to the short-lived nature of the Gag clone and development of HIV escape variants to the Nef clone, but did find that the infusion was safe and well tolerated. [8,11] Another small study in 3 patients on anti-retroviral therapy (ART) but with low level viremia established the safety and tolerability of escalating doses of gag-specific CTL clones of up to 3.3 x 10^9 /m2.[12] Transferred HIV-specific CTLs migrated to lymph nodes where they co-localized with HIV RNA+ cells in the parafollicular regions. The presence of CTL clones in the peripheral blood correlated with a significant but transient reduction in the number of HIV infected peripheral CD4+ T cells. These studies established the feasibility and safety of adoptive transfer of HIV-specific CTL clones but showed only modest efficacy in reducing HIV-load. Notably, these studies included actively viremic patients, and infused cells were generally monoclonal, had been expanded extensively in vitro and, hence, were likely highly differentiated. In a more recent study, HIV-specific CD8+ T cell clones derived from the central memory population of chronically HIV-infected patients on suppressive ART were expanded in vitro, and following autologous re-infusion could be detected for up to 84 days in blood and rectal tissue and retained or re-expressed memory markers (CD28+, CD62L+) and function (secretion of IL-2 and proliferation on cognate Ag exposure).[13] Virologic outcomes were not assessed in this study, however. In contrast to the latter study, we propose in this study to generate polyclonal CD4+ and CD8+ T cells specific for multiple HIV epitopes in multiple antigens. Among these studies and others with HIV-specific T cells, the only adverse events reported were mild with transient fever, arthralgias, myalgias, chills, and rigors following the infusions. Some of these events were associated with the administration of exogenous IL-2 given in conjunction with T cell infusion.[14,15]

It should be noted that there is also experience using gene modified autologous T cells in people living with HIV. For example, patients have been infused with CD4 T cells in which the CCR5 gene was rendered permanently dysfunction by a zinc-finger nuclease and the procedure was shown to be safe.[16] However, no sustained impact on CD4 T cells was achieved. Engineered antigen-specific T cells have also been employed in the context of multiple myeloma.[17] And CD19 CAR T cells have been used in the context of autologous transplantation in poor-risk relapsed and refractory B cell non-Hodgkin lymphoma.[18]

1.2.2. Safety of HIV-Specific T-Cells (HXTCs) Targeting gag, pol and nef

A study conducted in a collaboration between Children's National Health Systems and University of North Carolina (IND 15984) hypothesized that targeting gag, pol and nef simultaneously in a single CD4+ and CD8+ T cell product (HXTCs) would be safely tolerated in patients that have been on stable ART regimen with undetectable viral load based on the rationale that earlier studies demonstrated the safety of infusing CTLs in higher risk groups, including viremic and HIV patients with advanced immunosuppression. This was a Phase 1 proof of concept study (NCT02208167) of the administration of HXTCs to HIV-infected participants with undetectable plasma viremia (less than 50 copies/mL) on stable combination ART. Autologous T cells were stimulated *ex vivo* with autologous dendritic cells pulsed with a consensus Gag, Pol and Nef peptide mix to reflect the high sequence diversity of the HIV-1 virus. T cell expansion was performed in the presence of indinavir and raltegravir to inhibit HIV replication in cultures. After several rounds of stimulation (24–26 days), *ex vivo* expanded HXTCs showed a mean expansion of 146-fold

(range: 37–287). Participants meeting eligibility criteria received two HXTC infusions 2 weeks apart. Each HXTC dose was $2x10^7$ cells/m². Additionally, all participants underwent two leukapheresis procedures to measure the frequency of resting cell infection by the Quantitative Viral Outgrowth Assay (QVOA), at baseline and 12 weeks after the second HXTC infusion. All participants remained on ART throughout the study, classified by the stage of HIV infection stage during which treatment was initiated. Treatment during acute HIV infection was defined as starting ART within 30 days of the first positive HIV test identifying acute HIV infection.

Six participants enrolled and completed the study protocol consisting of two HXTC infusions (2 × 10e7/m2/dose) 2 weeks apart, without dose-limiting toxicity. Overall, the cell infusions were safe and well tolerated. Two participants (HXTC-02 and HXTC-07) had transient, self-limiting fevers and myalgias of DAIDs toxicity grade 1 severity 1-day post-infusion (HXTC-02) and 2 months post-infusion (HXTC-07). One participant (HXTC-07) also experienced a transient increase in viremia above the 40 copies/mL limit of detection 1 month following infusion, with a peak detectable viral load of 80 copies/mL that resolved without intervention within 60 days. No other treatment-related adverse events occurred. Other adverse events recorded were not attributable to the HXTC infusion. This included a transient increase in blood pressure with the leukapheresis procedure in four of six participants, an expected and well-described effect of the procedure, all at or below grade 3. We also determined whether the antiviral activity of CD8 T cells isolated from PBMCs of participants was altered following HXTC infusion using a viral inhibition assay (VIA). Baseline antiviral activity varied among participants and ranged from modest reduction of p24 production, to 50% of that seen in the absence of CD8 T cell addition, to no reduction.

To measure the antiviral activity in an assay with potentially more clinical relevance for participants on ART, during which time HIV antigen exposure is likely rare and at very low levels, we performed latency clearance assays (LCAs). In two of the six participants, the frequency of latent virus infection was too low at baseline to perform this assay with the cells available. The participant with the most improved CD8 performance in the VIA (HXTC-02) also showed an improved activity of CD8 T cells in the LCA following HXTC infusion, from modest (50%) reduction in virus recovery with co-culture with pre-infusion CD8 T cells to complete ablation of virus recovery with co-culture with post-infusion CD8 T ($p = 0.01$ by Fisher's exact test). Although a trend toward reduction was seen for the other three participants, it was not statistically significant due to the low number of positive wells in the control condition (three or fewer positive wells in control condition). The total number of wells plated was restricted due to limited cell availability and the large number of cells required for the assay, a known limitation of the assay, preventing definitive conclusions from being drawn. The week 13 post-infusion time point evaluated was chosen based on cell availability from the leukapheresis procedure; earlier post-infusion time points were not assessed.[14]

Thus harvest, expansion, and reinfusion of HXTCs from ART-treated, aviremic HIV-infected participants appears safe and well-tolerated. AEs were minimal and typically associated with the leukapheresis and the infusion of cryopreserved T-cells. HXTC infusion did not induce clinically perceptible signs of immune activation perhaps due to the low HIV antigen burden expressed during ART. Evaluations of the virological and immunological impact of HXTC infusion in this study are ongoing. Hence, this study demonstrated the safety of the HIV-specific T cell product

alone. Further, the data suggest that HXTCs confer a measurable augmentation of HIV-specific immune response and provide the rationale for a study to enhance the HIV-specific T cell response when administered to HIV+ individuals during the period of lymphopenia following high dose chemotherapy and ASCT. In a series of experiments in mouse models, Dr. Brad Jones has data that suggest that after cytotoxic chemotherapy, infused T cells in murine models are much more likely to persist and expand (personal communication). The importance of the timing of T lymphocyte infusion relative to lymphodepleting therapies has been emphasized previously in other contexts.[19]

1.2.3. Manufacturing and Preliminary Data on Ex-vivo Expanded multi-HIV antigen (gag, pol and nef) -specific T-cells Targeting Conserved Epitopes of Gag and Pol (HST-NEETs)

For this study, HST-NEETs will be manufactured in a process similar the one used for the HXTC products. Only differences are: 1) No use of the artificial K562 cells; and 2) the unique peptide sequences used for gag and pol targeting conserved epitopes. The nef pepmixes are the same as used for the HXTC product manufacture and include a pool of 150 15meric peptides derived from nef polyprotein designed to cover the high sequence diversity of the HIV-1 virus. For gag and pol pepmixes, the peptide cocktails are enriched for conserved/protective epitopes using a bivalent mosaic of peptides to cover the most common escape variants. The gag and pol pepmixes comprise 402 15meric peptides spanning the tHIVconsvX immunogen.[20] tHIVconsvX is a bivalent mosaic of peptides (two versions of each peptide to cover common escape variants) which spans only conserved regions of HIV and is further selected for epitopes that are associated with protection in natural HIV infection. HIV-infected individuals have previously been vaccinated with this tHIVconsvX in a prime/boost strategy using a ChAd.HIVconsv prime and a MVA.HIVconsv boost (BCN01 vaccine trial - NCT01712425).

1.3. NHL and Hodgkin Lymphoma in Patients with HIV Infection

Non-Hodgkin lymphoma (NHL) is an AIDS-defining diagnosis for patients infected with the Human Immunodeficiency Virus (HIV). While the incidence of NHL has decreased amongst HIV-infected patients since the advent of anti-retroviral therapy (ART), lymphoma remains a significant cause of death in this patient population.[21,22,23] Most patients with AIDS-related lymphoma have B-cell malignancies that include common entities such as diffuse large B-cell lymphoma, Burkitt lymphoma and Hodgkin lymphoma as well as less common clinical entities such as primary effusion lymphoma, plasmablastic lymphoma and primary central nervous system lymphoma.[24]

The prognosis for patients with AIDS-related lymphoma significantly improved in the era of ART therapy. Recent experience reported from the AIDS Malignancy Consortium (AMC) reports a 1-year event free survival of 83% in diffuse large B cell lymphoma (DLBCL) treated with DA-EPOCH-R.[25] The outcome for HIV-infected patients with DLBCL is now similar to the outcome for patients without HIV infection.[23,25]

While Hodgkin lymphoma is not an AIDS-defining diagnosis, the incidence of this disease is increased among patients with HIV infection.[23] Hodgkin lymphoma occurs more frequently in HIV infected patients than the general population with a standardized risk ratio of 14.7 (95% CI,

11.6-18.2) and is the third most common non-AIDS-defining malignancy in patients with HIV infection.[26] The survival for this group of patients also improved in the ART era. In patients with advanced disease (N=34), the 2-year PFS was 87% in a recently completed AMC trial of brentuximab-AVD. This is equivalent to HIV negative patients who develop Hodgkin lymphoma.

1.4. ASCT for Patients with HIV Infection

The prognosis for patients with refractory and relapsed NHL is poor with overall survival rates of less than 20% for patients treated with non-transplant salvage therapies. Based upon a randomized trial and numerous phase II trials, high-dose therapy with ASCT has been established as the standard of care for patients with chemotherapy-sensitive relapsed non-Hodgkin lymphoma.[27,28,29]

A trial published by the AMC enrolled 27 patients with either AIDS-related NHL or Hodgkin lymphoma in the setting of HIV infection.[30] Twenty patients, including 15 with NHL and 5 patients with Hodgkin lymphoma, subsequently underwent autologous HCT using a preparative regimen that consisted of dose-reduced busulfan and cyclophosphamide. One patient died of regimen-related toxicity. At a median follow-up time of 23 weeks, 10 patients were alive and free of disease.

The Blood and Marrow Transplant Clinical Trials Network (BMT CTN) 0803/AMC 071 trial was a multicenter phase 2 study of ASCT for patients with HIV-related lymphoma (HRL) that further demonstrated ASCT to be a safe and effective therapy for patients with HRL who meet standard transplant criteria.[1] Forty-three patients enrolled on this multi-institutional Phase II study, and forty underwent autologous transplantation after undergoing conditioning with the BEAM preparative regimen. Three patients had disease progression before conditioning and did not proceed to transplantation. At a median follow up of 24.8 months, the 1-year and 2-year overall survival rates were 87.3% and 82%, respectively. This compared favorably to the 1-year overall survival of 87.7% in a CIBMTR control cohort. The estimated risk of 1-year transplant related mortality (TRM) was 5.2%. The hazard ratio for TRM in the HIV-infected patient group compared to the CIBMTR control group was 1.05 (95% CI, 0.25-4.47; P = .9428).

1.5. HIV Infection and Risk of Opportunistic Infection following ASCT

Prior to the advent of ART, opportunistic infection was a key limitation to the use of ASCT for patients with HIV-related lymphoma. In the evolving transplant experience in the era of ART, opportunistic infection has not proven to be a significant limitation to the use of high-dose therapy. In the City of Hope group, Gabarre et al and AIDS Malignancy Consortium trial, CMV reactivation was noted in 3, 2 and 4 patients, respectively.[31,32]

A key finding in the transplant experience to date is that, with availability of ART, uncontrolled HIV infection is not a significant transplant-related complication. Even amongst those groups in which ART therapy was withheld on a planned basis during the peri-transplant period, most patients were able to achieve suppression of HIV to undetectable levels by the end of the first post-transplant year.[31] Gabarre et al. noted marked viral suppression in evaluable patients by 2 years post-transplant while 82.6% of patients enrolled on CTN 0803/AMC 071 had undetectable viral loads by Day+365.[33]

CD4 reconstitution following transplant appears comparable between groups. The City of Hope group, the AIDS Malignancy Consortium trial and Gabarre, et al all have noted a decrement in the CD4 count occurs through the 3rd transplant month, followed by a progressive rise in median CD4 counts through the first and second post-transplant years.[34] Alvarnez et al. demonstrated that the median CD4 counts returned to pretransplant levels by +Day 60 and remained at pretransplant levels through Day+365.[1]

1.6. Plasma DNA Tumor Markers

Over the past several years, several studies have demonstrated the potential for non-invasive "liquid biopsies" in the diagnosis and monitoring of cancer.[34] Plasma cell-free DNA (cfDNA) is double stranded, fragmented and highly labile.[35] NHL and HL patients have relatively high levels of tumor-derived DNA in cfDNA, referred to as ctDNA[36,37] compared to very low levels (<0.5% variant allele frequency, VAF) in solid-organ malignancies.[38] Mutations, including copy number abnormalities (aneuploidy), found in ctDNA in HL mirror those found in tumor biopsies.[38,39,40] Another sensitive tumor-derived marker found in ctDNA in lymphoma patients is clonal immunoglobulin (cIg).[41] There is similar sensitivity of cIg in HIV-associated lymphomas, including HL. [42,43] This approach has promise to identify high risk patients with residual disease or to identify recurrence of disease after relapse.

1.7. Rationale for the Current Study and Design

The experience with high dose therapy in patients with HIV and lymphoma is very encouraging with regard to tumor outcomes and safety. The investigators believe that ASCT may also provide a platform for interventions that might impact HIV disease and provide insights into cure strategies. The use of HST-NEETs is one such strategy. As noted above, the autologous transplant setting provides an opportunity in which hematopoietic space can be created by the preparative regimen and the persistence and efficacy of HIV-specific T cells can be best tested. With this in mind, we seek to assess the effect of these T cells on the long-term retroviral reservoir. In the past, the standard approach to assessing this reservoir was the viral outgrowth assay. The assay requires a very large blood draw and is labor intensive, requiring in vitro culture for 2-3 weeks. In addition, evidence has emerged that the assay misses a large part of the reservoir. A new assay, the intact proviral DNA assay (IPDA), is more sensitive and does not require a large volume blood draw or in vitro cultures.[44] Furthermore, it does not miss cells that may harbor intact HIV proviral DNA, but which fail to reactivate in the viral outgrowth assay. We will use the IPDA to assess the HIV-reservoir in this study, measured in copies of intact proviruses per million CD4 cells. The overall rationale for the study is that HIV-specific T cells infused before engraftment may be most likely to expand and persist. We note that some studies have monitored total proviral DNA[45]. The IPDA assay as implemented here will also yield measurement of total proviral DNA. The HST-NEETs may be especially efficient at targeting HIV antigens. By infusing T cells at the time when hematopoietic space has been created and measuring the impact on the HIV reservoir, we should gain insight into whether the general strategy of adoptive immunotherapy targeting HIV is worth pursuing.

CHAPTER 2

2. STUDY DESIGN

2.1. Study Overview

Depending on their ALC results, eligible participants will have 100-120 mL of peripheral blood or 80-100 mL of MNCs via apheresis collected and shipped to Children's National Hospital at ambient temperature. The sample will be used to manufacture the HST-NEET product. The autologous peripheral blood stem cell graft suitable for rescue following conditioning will be obtained either before or after the collection to generate HST-NEETs. Pre-transplant conditioning will consist of BEAM; BCNU 300 mg/m^2 on Day -6, Etoposide 100 mg/m^2 BID and Ara-C 100 mg/m^2 BID on Days -5, -4, -3 and -2 and Melphalan 140 mg/m^2 on Day -1. ASCT on Day 0. If the mobilized graft contains greater than 5.0 x 10^6 CD34+ cells per kg, any additional cells should be cryopreserved as a "back-up" graft in the event of graft failure related to the HST-NEETs. Participants will receive one dose (2 x 10^7 cells/m^2) of HST-NEETs between Days +3 to +7 based on the clinical condition of the participant (as outlined in Section 2.6). If this window is missed, the HST-NEETs may be administered up to Day +30 post-ASCT. Participants will be followed for at least one year after ASCT.

2.2. Hypothesis and Specific Objectives

2.2.1. Primary Hypothesis

Following high dose chemotherapy and ASCT for participants with HIV-associated lymphoma, infusion of T cells targeting multiple HIV antigens (HST-NEETs) within 1 week will be feasible and these cells will persist and deplete the intact HIV proviral reservoir.

2.3. Study Objectives

2.3.1. Primary Objective

The primary objective is to determine both the proportion of participants receiving ASCT who were able to be treated with HST-NEETs within 1 week of ASCT in a cooperative multi-institutional setting and the efficacy of infused HST-NEETs in reducing the HIV intact proviral reservoir at 6 months after ASCT.

2.3.2. Secondary Objectives

The secondary objectives are to assess:

1. Progression-free survival at 6 months and 1-year post-ASCT;
2. The incidence and severity of acute infusion-related toxicities;
3. The impact of therapy on the HIV intact proviral reservoir at 1-year post-ASCT.

2.3.3. Exploratory Objectives

The exploratory objectives are to assess:

1. CR and CR+PR rates at Day 100 post-ASCT;
2. Overall survival at 6 months and 1-year post-ASCT;
3. Time to hematopoietic recovery;
4. Incidence of infections through 1-year post-ASCT;
5. Non-relapse mortality at 6 months and 1-year post-ASCT;
6. Toxicities through 1-year post-ASCT;
7. Assessment of plasma DNA in blood (clonal Ig DNA) as a tumor marker at Day 100, 6 months and 1-year post-ASCT;
8. Impact of therapy on the HIV intact proviral reservoir at Day 100 post-ASCT;
9. HIV RNA in plasma at Day 100, 6 months, and 1-year post-ASCT;
10. Impact of therapy on the total proviral HIV DNA at Day 100, 6 months, and 1-year post-ASCT;
11. HST-NEETs persistence and expansion *in vivo.*
12. The time from transplant until relapse/progression of the primary disease, or initiation of anti-lymphoma therapy. Death in the absence of relapse/progression will be considered a competing risk.

2.4. Participant Eligibility

Participants must meet specified eligibility criteria for entry into the study.

2.4.1. Participant Inclusion Criteria

Participants fulfilling the following criteria will be eligible for entry into this study:

1. Age 15 years old or older at time of enrollment.
2. Receiving antiretroviral therapies (ART) with HIV viral load < 200 copies or below the limit of detection by standard commercial assay. An HIV-1 RNA measurement that is ≥ 200 copies measured by an FDA-approved commercial assay but <500 copies may be allowed if this is followed by an HIV-1 RNA measurement below the limit of detection or if an exception is approved.
3. Diagnosis of diffuse large B-cell lymphoma, composite lymphoma with greater than 50% diffuse large B-cell lymphoma, mediastinal B-cell lymphoma, immunoblastic, plasmablastic, Burkitt or high-grade B cell lymphoma or classical Hodgkin lymphoma. Participants with aggressive B-cell lymphoma that is transformed from follicular lymphoma are eligible for the study, pending fulfillment of other criteria.
4. Plan to treat participant with high dose chemotherapy and autologous hematopoietic stem cell transplantation (ASCT).

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5. All participants must have chemosensitive disease as demonstrated by at least a partial response (as defined by the criteria in Chapter 3) to induction or salvage therapy.
6. Absolute Lymphocyte Count (ALC) greater than or equal to 800/µL. If ALC is less than 800/uL but still above 400/uL patient may be eligible if the HST-NEETs manufacturing blood product is collected via non-mobilized leukapheresis(apheresis).
7. Participants with adequate organ function as measured by:
    a) Cardiac: Participants must have a left ventricular ejection fraction at rest greater than or equal to 40% demonstrated by MUGA or echocardiogram.
    b) Hepatic:
        i. Bilirubin less than or equal to 2.0 mg/dL (except for isolated hyperbilirubinemia attributed to Gilbert syndrome or antiretroviral therapy as specified in Appendix D) and ALT and AST less than or equal to 3x the upper limit of normal.
        ii. Concomitant Hepatitis: Participants with chronic hepatitis B or C may be enrolled on the trial providing the above criteria are met. In addition, they must not have evidence of active viral replication by PCR, and no clinical or pathologic evidence of irreversible chronic liver disease.
    c) Renal: Creatinine clearance (calculated creatinine clearance is permitted based on institutional practice) greater than 40 mL/min.
    d) Pulmonary DLCO (corrected for hemoglobin), FEV1, FVC greater than or equal to 45% of predicted.
8. Voluntary written consent or assent obtained prior to enrollment on study with the understanding that consent or assent may be withdrawn by the participant at any time without prejudice to future medical care.

2.4.2. Participant Exclusion Criteria

Participants with the following will be ineligible for registration onto this study:

1. Karnofsky performance score less than 70%.
2. Participant is known to have an HIV subtype other than B.
3. Participant has documented raltegravir or protease inhibitor resistance.
4. Myocardial infarction within 6 months prior to enrollment or New York Heart Association (NYHA) Class III or IV heart failure, uncontrolled angina, severe uncontrolled ventricular arrhythmias, or electrocardiographic evidence of acute ischemia.
5. Uncontrolled bacterial, viral or fungal infection (currently taking medication and with progression or no clinical improvement).
6. Participant has active CNS involvement.

7. Participants with prior malignancies except resected non-melanoma skin cancer or treated cervical carcinoma in situ. Cancer treated with curative intent greater than or equal to 5 years previously will be allowed. Cancer treated with curative intent less than 5 years previously may be eligible must be reviewed and approved by the Protocol Officer or Chairs.
8. Female participants that are pregnant as per institutional definition or breastfeeding.
9. Fertile men or women unwilling to use contraceptive techniques from the time of initiation of mobilization until six-months post-transplant.
10. Prior autologous or allogeneic HCT, or prior therapy with chimeric antigen receptor (CAR) T-cells.
11. Participants with evidence of MDS/AML or abnormal cytogenetic analysis indicative of MDS on the pre-transplant bone marrow examination. Pathology report documentation need not be submitted.
12. Steroids greater than 0.5 mg/kg/day prednisone equivalents.
13. Bone marrow involvement by lymphoma at time of workup. Prior history of bone marrow involvement is allowed if cleared prior to ASCT.

2.4.3. Eligibility for Conditioning Regimen and Transplant

Participants must meet the criteria specified above prior to enrollment. Additionally, the participant must meet the following (eligibility) criteria for conditioning and ASCT:

1. Procurement of an autologous peripheral stem cell graft judged adequate by institutional standards (recommended target is greater than or equal to 2.0 x 10^6 CD 34^+ cells/kg) or if PBSC mobilization fails, cells can be obtained by bone marrow harvest per institutional practices (in cases where bone marrow will be used for transplantation, the required CD34+ dose does not apply and institutional requirements for total nucleated cell dose should apply). If the mobilized graft contains greater than 5.0 x 10^6 CD34+ cells per kg, any additional cells should be cryopreserved as a "back-up" graft in the event of graft failure related to the HST-NEETs.
2. Within 10 days prior to conditioning regimen, participants should be assessed for adequate organ function, as measured by:
    a) Hepatic: Bilirubin less than or equal to 2.0 mg/dL (except for isolated hyperbilirubinemia attributed to Gilbert syndrome or antiretroviral therapy as specified in Appendix D) and ALT and AST less than or equal to 3x the upper limit of normal.
    b) Renal: Creatinine clearance (calculated creatinine clearance is permitted based on institutional practice) greater than 40 mL/min.

2.5. Treatment Plan

Once enrolled, the Cellular Therapy Laboratory (CTL) at Children's National Hospital (CNH) should be contacted by the site at CTL@childrensnational.org at least 2 weeks priors to collection

to confirm a date for shipping. The site will collect 100-120 mL of peripheral blood or 80-100 mL of MNCs via apheresis to be shipped at ambient temperature to the manufacturing center and used to generate the HST-NEET product. This collection will need to occur at least 6-8 weeks prior to ASCT due to the time required to manufacture the HST-NEETs. Should the initial production of the HST-NEETs product be unsuccessful, the BMT CTN Data Coordinating Center (DCC) staff and clinical site study team will be notified, and a second collection and manufacturing attempt may occur pending feasibility. Details on shipping will be provided in the Appendix C. However, treatment with high dose chemotherapy and autologous peripheral blood stem cell transplantation should NOT be delayed for the sole purpose that the patient participates on this trial.

The immediate pre-ASCT evaluation will be carried out according to the operating procedures of the participating institutions and should be in keeping with the data reporting requirements of this study. All participants enrolled on this protocol will be hospitalized in accordance with the procedures for recipients of ASCT as defined by the treating institutions. Table 2.5 below describes the conditioning regimen (BEAM) for this study. Transplant day is referred to as Day 0. Treatment plan activities prior to or after Day 0 are denoted as Day minus or Day plus, respectively.

TABLE 2.5: BEAM + HCT REGIMEN*

Day							
-6	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**+3-+7**
BCNU 300 mg/m^2	Ara-C 100 mg/m^2 BID VP-16 100 mg/m^2 BID	Ara-C 100 mg/m^2 BID VP-16 100 mg/m^2 BID	Ara-C 100 mg/m^2 BID VP-16 100 mg/m^2 BID	Ara-C 100 mg/m^2 BID VP-16 100 mg/m^2 BID	Melphalan 140 mg/m^2	HCT	HST-NEETs 2x10^7 cells (single dose)

***Conditioning Regimen Administration Schedule:** The conditioning regimen administration schedule may be modified ± 1 day according to institutional practice. Day 0 will be the day of HCT.

1. **Carmustine (BCNU):** 300 mg/m^2 on Day –6, to be administered intravenously per institutional guidelines.
2. **Cytarabine (Ara-C):** 100 mg/m^2 BID on Days –5 through –2, for a total of 8 doses, to be administered intravenously per institutional guidelines.
3. **Etoposide (VP-16):** 100 mg/m^2 BID on Days –5 through –2, for a total of 8 doses, to be administered intravenously per institutional guidelines.
4. **Melphalan:** 140 mg/m^2 on Day -1, to be administered intravenously per institutional guidelines.
5. **Autologous graft**: Hematopoietic progenitor cells will be cryopreserved according to the institutional standards and administered post thaw on Day 0. If the mobilized graft contains

greater than 5.0 x 10^6 CD34+ cells per kg, any additional cells will be cryopreserved as a "back-up" graft in the event of graft failure related to the HST-NEETs.

6. **HST-NEETs: One dose** (2 x 10^7 cells/m^2) should be given between Days+3 to +7, or at the earliest time participant is deemed able to receive the cells based on clinical assessment of the participant as described in Section 2.6.1.

2.6. HST-NEETs – Eligibility, Dosage, and Administration

2.6.1. Eligibility to Receive HST-NEETs

HST-NEETs should be administered between Day +3 and Day +7 post-ASCT. Prior to administration, the site investigator should evaluate the participant's condition to receive the infusion. The following criteria must be met at the time of infusion:

- No active or uncontrolled infections
- Participant must be hemodynamically stable

If HST-NEET infusion is not feasible between Day +3 and Day +7, the participant may be re-assessed and the infusion occur through Day +30 post-ASCT.

2.6.2. HST-NEETs Dosage

Participants will receive $2x10^7/m^2$ cells as a single intravenous (IV) infusion. The cells will be cryopreserved ideally at $1x10^7$ T cells per mL but up to $5x10^7$ cells/mL.

2.6.3. HST-NEETs Administration

HST-NEETs will be manufactured from peripheral blood or apheresis collection, stored and maintained in the Children's National Hospital (CNH) Cellular Therapy Laboratory (CTL) until shipment to the enrolling institution. The cryopreserved product will be shipped to the study site from Children's National Hospital in a Liquid Nitrogen Dry Shipper via overnight shipping to arrive by Day -7. Prior to releasing the HST-NEETs to the site, the manufactured product will undergo viability testing, endotoxin testing, sterility testing (aerobic, anerobic and fungal), mycoplasma testing, phenotyping, alloreactivity testing and HLA typing. In the case of HLA typing, the HLA typing of the final drug product will be compared to the HLA typing of the participant to ensure the correct identity of the drug product. On day of infusion the study site will then thaw the cryovial(s) containing the product, transfer to a sterile syringe, and administer to the participant. The cryovial(s) will be cultured locally to assess product sterility at time of administration. Specific instructions will be provided to all study sites regarding the procedure for thawing and administration of the product. In summary, the following should occur:

- The HST-NEETs product will be reviewed prior to administration by two licensed health care providers per institutional standards. This review should confirm that the identity of the cryovial/syringe matches the prescription for infusion, as well as a visual inspection of the cryovial/syringe.

- The participant will receive Diphenhydramine (Benadryl®) and Acetaminophen (Tylenol®) as pre-medications or equivalent per institutional guidelines prior to HST-NEET infusion. Steroids should be avoided given their detrimental effect on the survival of the infused virus specific T cells
- Cell Administration: HST-NEETs will be given by intravenous infusion over 1-10 minutes through a peripheral or central line.
- The infusion will be given under supervision by a transplant physician. The participant will be monitored according to below:
    - Participants should remain on continuous pulse oximetry for at least 30 minutes post-infusion.
    - Vital signs should be monitored pre-infusion, at the end of infusion and at 30- and 60-minutes post infusion.
    - Acute toxicities should be reported up to 24 hours after infusion. For outpatients, this may be accomplished by self-report or report from a caregiver.
- Participants will receive supportive care for acute toxicity directly attributable to HST-NEETs as appropriate (See Appendix E). Steroids, though contraindicated, can be administered if there are life-threatening anaphylactic reactions (See Appendix E).

2.6.4. Duration

All transplanted participants will be followed for 1-year post ASCT. A time period of 28 days from infusion of HST-NEETs will constitute the time for clinical safety monitoring specific to HST-NEETs.

2.7. Supportive Care

All supportive care will be given in keeping with local institutional guidelines.

2.7.1. Corticosteroids

As steroids will have a detrimental effect on the survival of the infused virus specific T cells they should be avoided, if possible, for the first 6 weeks following infusion. If the subject has a clinical indication for steroid use such as CRS or engraftment syndrome steroids may be administered at 1-2mg/kg/day and then weaned/discontinued as soon as possible.

2.7.2. Growth Factors

All participants will receive G-CSF 5-10 mcg/kg/day or per institutional guidelines, beginning no sooner than 24 hours following the infusion of HST-NEETs or no later than Day +7 post-transplant in participants not receiving HST-NEETs and continuing until an ANC greater than or equal to 500/mm^3 is obtained for 2 consecutive days.

2.7.3. Blood Products

Transfusion thresholds for blood product support will be consistent with standard institutional guidelines. All blood products will be irradiated.

2.7.4. Prophylaxis Against Infections

All participants will receive prophylaxis against bacterial, fungal and viral infections during the post-ASCT period according to institutional guidelines. Additional guidelines for HIV participants in this study are summarized in Appendix D.

2.7.5. Post-HCT Lymphoma Therapy

Consolidative localized radiation therapy (maximum 3 sites) is allowed to areas of previous bulk disease (greater than 5 cm). Localized radiation should be completed by Day 100 post-ASCT. No other anti-lymphoma therapy is allowed in the post-transplant setting or the participant will be considered to have progressed.

2.7.6. Anti-Retroviral Therapy (ART)

Anti-retroviral (ART) therapy is only effective when administered in combination and with consistency. Single agent therapy or repeated interruptions in therapy lead to resistance. The ART guidelines below are designed to minimize the possibility of drug interactions with high dose chemotherapy and to administer ART only when it is anticipated that the ART regimen can be consistently complied with (i.e., preparative regimen toxicities such as nausea and mucositis will not interfere with dosing schedules). A few ARTs require special mention as discussed below.

Zidovudine (AZT):
AZT is myelosuppressive and should not be used in the ART regimen administered after ASCT.

Efavirenz:
Efavirenz has a long half-life and resistance develops especially rapidly when it is administered in the absence of other ARTs. When an efavirenz containing regimen is stopped, levels of other ARTs will fall much more rapidly than those of efavirenz resulting in the functional equivalent of single agent therapy and risking resistance. In order to avoid this risk, ideally at least 4 weeks prior to autologous transplant the participant should be switched to a different triple drug regimen without efavirenz. Approximately 2 to 3 months post ASCT the participant can revert to previous regimen if wished.

Protease inhibitor-based regimen, or a regimen boosted with either ritonavir or cobicistat:
Protease inhibitor-based regimen, or a regimen boosted with either ritonavir or cobicistat contain ARTs that have a strong potential to inhibit CYP3A4. These regimens would inhibit a route of elimination and may cause the substrate drug (e.g., VP-16) to increase to a potentially supra-therapeutic or toxic level, resulting in the need to decrease the dose of the substrate. Since the pre-treatment conditioning regimen should not be dose-adjusted, in order to avoid this risk, ideally at least 2 weeks prior to autologous transplant the participant should be switched to a different triple

drug regimen. Approximately 2 to 3 months post ASCT the participant can revert to previous regimen if wished.

Patients on established antiretroviral regimens:

1) If patient is on AZT containing regimen, efavirenz containing regimen, a protease inhibitor-based regimen, or a regimen boosted with either ritonavir or cobicistat, change ART to an alternative medicine (such as an integrase inhibitor) prior to starting conditioning.

2) In the rare cases of chemotherapy induced mucositis where no oral medications can be taken, then ART should be discontinued. In this case, weekly HIV RNA levels should be checked to monitor for HIV breakthrough.
3) Resume an ART regimen as soon as participant can tolerate the total ART regimen as mucositis has improved sufficiently to resume PO.

2.8. Participant Risks

2.8.1. Therapy Toxicities

All toxicities will be graded using the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. Please refer to www.fda.gov for full adverse event information regarding the commercially available conditioning regimen agents listed below.

2.8.2. Carmustine (BCNU)

Carmustine is an alkylating agent.
Carmustine side effects include:

- Cardiovascular: tachycardia, chest pain
- Cutaneous: alopecia, rash, skin burning/swelling/erythema
- Gastrointestinal: nausea, vomiting, diarrhea, anorexia, constipation, abdominal pain, GI bleeding
- Hematologic: leukopenia, thrombocytopenia, anemia, bleeding
- Hepatic: hepatotoxicity
- Neurologic: seizure, encephalopathy, headache, aphagia, depression, stroke, fluid leakage from brain/spinal cord, intracranial hemorrhage
- Renal: renal toxicity
- Respiratory: pneumonitis, interstitial lung disease, pulmonary fibrosis
- Miscellaneous: lethargy, infection, infusion reaction, allergic reaction including anaphylaxis, secondary malignancy

2.8.3. VP-16 (Etoposide)

VP-16 is a semi-synthetic podophyllotoxin derivative. Etoposide side effects include:

- Cardiovascular: hypotension
- Cutaneous: alopecia, rash, itching, Stevens-Johnson Syndrome
- Gastrointestinal: nausea, vomiting, diarrhea, anorexia, abdominal pain, mucositis, difficulty swallowing
- General: lethargy
- Hematologic: leukopenia, thrombocytopenia, anemia
- Hepatic: Hepatotoxicity
- Neurologic: seizure, peripheral neuropathy
- Miscellaneous: infection, cortical blindness, optic neuritis, infusion reaction, allergic reaction including anaphylaxis, secondary malignancy

2.8.4. Cytarabine (Ara-C)

Cytarabine, commonly known as Ara-C, is a synthetic nucleoside. Cytarabine side effects include:

- Cardiac and vascular: thrombophlebitis, pericarditis, chest pain
- Cutaneous: photosensitivity, rash, itching, alopecia
- Gastrointestinal: nausea, vomiting, diarrhea, anorexia, ulcers, mucositis, rectal swelling, abdominal pain
- Hematologic: anemia, thrombocytopenia, leukopenia
- Hepatic: hepatotoxicity
- Neurologic: peripheral neuropathy (motor and sensory), dizziness, headache, Posterior Reversible Encephalopathy Syndrome (PRES), somnolence/drowsiness, personality changes, coma
- Pulmonary: interstitial pneumonitis
- Miscellaneous: back pain, conjunctivitis, infection, myalgia/myopathy, allergic reaction including anaphylaxis

2.8.5. Melphalan

Melphalan, an alkylating agent, is a phenylalanine derivative of nitrogen mustard. Melphalan side effects include:

- Cardiac and vascular: edema, heart failure, vasculitis
- Gastrointestinal: mucositis, nausea, vomiting, diarrhea
- General: fatigue
- Hematologic: anemia, thrombocytopenia, neutropenia
- Hepatic: abnormal liver function tests, hepatitis
- Pulmonary: shortness of breath, pulmonary fibrosis
- Renal: renal impairment
- Miscellaneous: allergic reaction including anaphylaxis, secondary malignancy

2.8.6. ASCT

ASCT recipients incur risks from high-dose conditioning and post-ASCT therapy, which must be weighed against the risk of the disease for which the ASCT is prescribed. Major risks following transplantation include: 1) Infection which can be bacterial, viral, parasitic, or fungal. Often, these infections are life-threatening, particularly when caused by viral or fungal agents, and are associated with high mortality in the transplant population. The recent published experience shows no increased risk of CMV viremia in HIV+ patients, and no increase in mortality related to that virus; 2) Damage of all or any of the major organs may occur as a result of reactions to drugs (e.g., chemotherapy, antibiotics, anti-fungal medications), and as a result of destructive processes (e.g., infection), and may have a fatal outcome; brain damage can result in severe loss of cognitive or neurologic function; 3) Relapse or progression of lymphoma may occur, especially in participants with advanced disease status at time of treatment; 4) Unknown toxicities may occur in any individual participant due to multiple events and cumulative effects which may involve any and all organs, including the brain; and, 5) Death.

2.8.7. HST-NEETs

The experience with T cell recipients as reported in the literature suggests that serious reactions to virus –specific T cell therapy are rare. Possible side effects of virus specific T cell therapy in participants with virus-associated malignancies and HIV include fever and flu-like symptoms. In the case of participants with EBV+ lymphoma, these symptoms, along with swelling of tumor sites, have occurred primarily in participants who have bulky disease due to lymphoma following infusion of T cells or in participants receiving genetically enhanced T-cells. Six participants enrolled and completed a study protocol consisting of two HIV-specific T cells (HXTC) infusions ($2 \times 10e7/m^2$/dose) 2 weeks apart, without dose-limiting toxicity (NCT02208167).[14] Overall, the cell infusions were safe and well tolerated. Two participants (HXTC-02 and HXTC-07) had transient, self-limiting fevers and myalgia of DAIDs toxicity grade 1 severity 1-day post-infusion (HXTC-02) and 2 months post-infusion (HXTC-07). One participant (HXTC-07) also experienced a transient increase in viremia above the 40 copies/mL limit of detection 1 month following infusion, with a peak detectable viral load of 80 copies/mL that resolved without intervention within 60 days. No other treatment-related adverse events occurred. Other adverse events recorded were not attributable to the HXTC infusion. This included a transient increase in blood pressure with the leukapheresis procedure in four of six participants, an expected and well-described effect of the procedure, all at or below grade 3. We also determined whether the antiviral activity of CD8 T cells isolated from PBMCs of participants was altered following HXTC infusion using a viral inhibition assay (VIA). Baseline antiviral activity varied among participants and ranged from modest reduction of p24 production, to 50% of that seen in the absence of CD8 T cell addition, to no reduction.

The HST-NEETs product in this study does not include genetically enhanced T-cells. As the HST-NEETs product is an autologous product, there should not be a risk of graft-versus-host disease (GVHD). The HST-NEETs product will undergo release testing for microbial contaminants. However, it is possible that undetected infectious agents could be administered with the HST-NEETs and cause fever, chills, hypotension, sepsis, or death. Since this is an autologous product, it is unlikely that novel infectious agents will be transferred to participants. None of these adverse

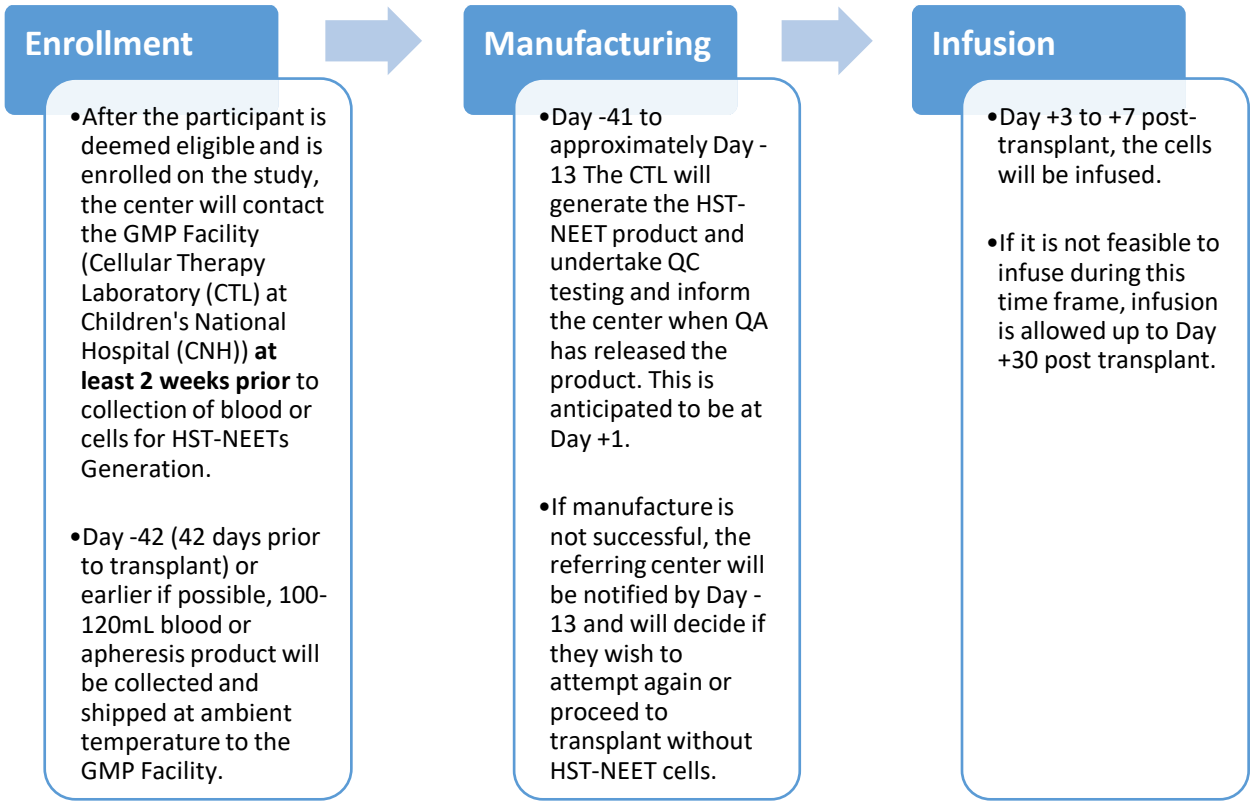
events (Aes) have been observed in studies of T cell therapy to date including our experience with the HXTC product (IND15984) which uses the exact same manufacturing SOP with the only difference being the gag and pol pepmixes used to pulse the antigen presenting cells ex vivo.

The planned HST-NEETs infusion is an immunotherapeutic product using HIV-infected participants' biological materials. Thus, this product will be handled as if infectious agents are present.

2.9. Investigational Product Supply

HST-NEETs product will be generated from 100-120 mL of recipient peripheral blood or 80-100 mL of MNCs collected via apheresis. These samples will be shipped in ambient temperature to the Cellular Therapy Laboratory at Children's National Hospital with shipping details provided in Appendix C. The HST-NEETs cell product is cryopreserved and shipped to the transplant centers for administration.

An overview of the Manufacturing Logistics is provided below.



Carmustine, etoposide, cytarabine and melphalan are commercially available agents and will not be provided by the study. Please administer commercially available medications per institutional standards, as described in Section 2.5, and store per package insert instructions

2.10. Study Conduct

This study will be conducted in accordance with the protocol, the BMT CTN Administrative Manual of Procedures (MOP), and the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki
- Applicable ICH Good Clinical Practice (GCP) Guidelines
- Applicable laws and regulations

The National Marrow Donor Program (NMDP) single Institutional Review Board (IRB) of Record will oversee this study and conduct the study-specific reviews as required by federal regulations and per the NMDP IRB Standard Operating Procedures (SOPs).

Site personnel will enter data in the electronic case report form (eCRF) in Advantage eClinical® as described in the BMT CTN 1903 Forms Guide. Source documentation should be made available for monitoring visits, audits and regulatory inspections as described in the BMT CTN MOP.

Participating Principal Investigators (Pis) bear ultimate responsibility for training of site staff as well as the scientific, technical, and administrative aspects of conduct of the protocol, even when certain tasks have been delegated to coinvestigators, sub-investigators, or staff. The Pis have a responsibility to protect the rights and welfare of participants and comply with all requirements regarding the clinical obligations and all other pertinent requirements in 21 CFR part 312. In addition to following applicable federal, state, and local regulations, investigators are expected to follow ethical principles and standards and receive training in GCP every three years and human subjects training within the past 3 years and thereafter as per institutional requirements.

CHAPTER 3

3. STUDY ENDPOINTS

3.1. Definition of Disease Status

Tests used for evaluation of disease status include physical examination, laboratory testing, bone marrow biopsy and aspirate, PET scans, and CT scans of neck, chest, abdomen and pelvis as indicated.

Imaging will be assessed at baseline prior to transplant and prospectively. The Lugano Classification[46] will be used to assess response after baseline in comparison to the baseline imaging. As outlined in Appendix B, lymphoma response is defined as any participant who does not progress on this study, including participants with active disease who achieve Complete Metabolic Response (CMR)/Complete Radiologic Response (CR), Partial Metabolic Response (PMR)/ Partial Remission (PR), or No Metabolic Response (NMR)/Stable Disease (SD) by PET/CT. Because the natural history of these high risk relapsed and refractory participants is progression (and often mortality), any response or even stable disease is an improvement over expected outcomes.

CT responses will be based on anatomical measurements of target/evaluable/new lesions.

The possible response outcomes are complete radiologic response (CR), partial remission (PR), stable disease (SD) or progressive disease (PD) as defined in Appendix B.

3.2. Primary Endpoints

3.2.1. Feasibility

Feasibility is defined as a participant receiving HST-NEETs within 1-week post-ASCT. All eligible and enrolled participants will be considered part of the feasibility assessment.

3.2.2. Efficacy

Efficacy will be measured by the reduction in intact proviral reservoir. This will be evaluated using the change in intact proviral DNA assay (IPDA) at enrollment through 6 months post-ASCT among participants with samples at both timepoints.

3.3. Secondary Endpoints

3.3.1. Progression-Free Survival

Participants are considered a failure for this endpoint if they die or if they relapse/progress or receive anti-lymphoma therapy, other than post-transplant consolidative localized radiation (maximum 3 sites) to sites of prior bulk disease pre-transplant (greater than 3cm). The time to this event is the time from transplant until death, relapse/progression, receipt of anti-lymphoma

therapy, or last follow up, whichever comes first. This will be assessed at 6 months and 1-year post-ASCT.

3.3.2. Acute Infusion Related Toxicity

Acute infusion related toxicities are defined as toxicities related to the infusion of HST-NEETs that occur within 24 hours of the infusion. Toxicities will be graded per CTCAE criteria v5.0.

3.3.3. Intact Proviral Reservoir

Impact on intact proviral reservoir will be assessed using the IPDA at 4-8 weeks prior to transplant and 12 months following ASCT.

3.4. Exploratory Objectives

3.4.1. CR and CR+PR Rate at Day 100 Post-ASCT

CR or PR will be assessed according to the LYRIC criteria at Day 100 post-ASCT (see Appendix B).

3.4.2. Overall Survival

Overall survival is defined as death from any cause within 6 months and within 1-year post-ASCT. Surviving participants will be censored at the date of last follow-up.

3.4.3. Time to Hematopoietic Recovery

Time to neutrophil recovery will be the first of three consecutive labs of greater than or equal to 500 neutrophils/μL following the expected nadir. Time to platelet engraftment will be the date platelet count is greater than or equal to 20,000/μL for the first of three consecutive labs with no platelet transfusions 7 days prior.

3.4.4. Incidence of Infections

The incidence of viral, fungal and bacterial infections will be tabulated. All Grade 2 and Grade 3 infections will be reported according to the BMT CTN technical guidelines from Day 0 up to 1-year post-transplant. Infections of interest will be captured and described. The incidence rate of infections is defined by the number of infections divided by the total person-time accumulated over the duration of the study.

3.4.5. Non-Relapse Mortality

Non-Relapse Mortality (NRM) is defined as death occurring in a participant without relapse progression and will be measured at 6 months and at. 1-year.

3.4.6. Toxicities

Toxicities related to the BEAM conditioning regimen and HST-NEETs infusion beyond the 24-hour acute toxicity monitoring period will be defined by using the version 5.0 CTCAE criteria. All grades of toxicity related to HST-NEETs will be collected. Only grade 3 or higher conditioning regimen related toxicities will be collected.

3.4.7. Ig DNA in Blood

Blood specimens will be collected prior to the initiation of conditioning, and at Days 100, 6 months and 1-year post-ASCT. The presence of clonal Ig DNA in plasma will be assessed at each of these time points.

3.4.8. Intact Proviral Reservoir at Day 100

Impact on intact proviral reservoir assessed using the IPDA Day 100 post-ASCT as compared to baseline at 4-8 weeks prior to transplant.

3.4.9. HIV RNA in Blood

HIV RNA in plasma will be measured by a sensitive investigational single copy assay (SCA, detection limit 0.38 copy/ml). Blood specimens will be collected, and plasma HIV RNA measured, at study visits corresponding to those associated with IPDA testing: 4-8 weeks prior to transplant, and at Days 100, 6 months and 1-year post-ASCT.

3.4.10. Total Proviral HIV DNA

This assay will be measured at study visits corresponding to those associated with IPDA testing: 4-8 weeks prior to transplant, and at Days 100, 6 months and 1-year post-ASCT. This assay will provide information about the proviral reservoir in patients where the IPDA fails due to sequence variation.

3.4.11. HST-NEETs Persistence and Expansion in Vivo

Persistence of HST-NEETs will be measured by frequency of HIV-1 antigen-specific (gag, pol, nef) CD8+ T-cells by ELIspot at baseline and post-infusion at timepoints at Days 100, 6 months, and . 1-year post transplant. Change in T cell responses from baseline to post-infusion, measured by frequency of cells secreting IFN-γ by multimer analysis and/or intracellular cytokine staining and/or ELIspot and/or TCR sequencing will be done depending on PBMC cell numbers available and reagent availability.

3.4.12. Relapse/Progression of Primary Disease

The time from transplant until relapse/progression of the primary disease, or initiation of anti-lymphoma therapy. Death in the absence of relapse/progression will be considered a competing risk.

CHAPTER 4

4. PARTICIPANT ENROLLMENT AND EVALUATION

4.1. Enrollment Procedures

4.1.1. Screening and Eligibility Procedures

Prior to collection of peripheral blood, or apheresis sample, needed to manufacture HST-NEETs, the transplant center will confirm participant eligibility as per section 2.4. Once confirmed, participants will be registered using the BMT CTN Electronic Data Capture System (Advantage eClinical®). An authorized user at the transplant center completes initial screening by entering participant demographics and Segment 0 Enrollment Form in Advantage eClinical®. The eligibility screening includes questions that will verify eligibility. If the participant is eligible, a participant study number will be generated. The participant will be consented to the conditioning regimen and ASCT per institutional standards.

4.1.2. Co-Enrollment

Patients may participate in observational studies; however, patients who are participating in an interventional study must be approved individually by the Study Chairs, Sponsor, and Protocol Officer in order to participate in this study. Participants on study should not participate in an interventional study during the . 1-year follow up period unless pre-approved.

4.2. Collection of Study Product Components

4.2.1. Collection of peripheral blood or apheresis product

Once enrolled, the Cellular Therapy Laboratory at Children's National Hospital will be notified by the site at CTL@childrensnational.org at least 2 weeks prior to collection to confirm a date for shipping. HST-NEETs product will be generated from 100-120 mL of recipient peripheral blood or from an 80-100mL apheresis product with a minimum of $1x10^9$ TNC, with >90% viability. This collection will need to occur at least 6 weeks prior to ASCT due to the time required to manufacture the HST-NEETs. Additionally, it should occur before the participant starts G-CSF or at least 1 week after their last dose of G-CSF. The participant's Absolute Lymphocyte Count should be ≥800/µL prior to blood draw for whole blood collection and ≥400/µL and <800/µL for apheresis collection. Should the initial production of HST-NEETs product be unsuccessful, the BMT CTN DCC and clinical study team will be contacted and have the opportunity to re-initiate the HST-NEETs cell line. These samples will be shipped in ambient temperature to the Cellular Therapy Laboratory at Children's National Hospital overnight on the day of collection as described in the Appendix C.

4.3. Study Monitoring

4.3.1. Follow-Up Schedule

The follow-up schedule for scheduled study visits is outlined in Table 4.3.1. A detailed description of each of the forms and the procedures required for forms completion and submission can be found in the BMT CTN 1903 eCRF Completion Guide. This guide is available on the homepage of the Internet data entry system.

Follow-up Visits: Follow-up visits will begin as soon as participants are enrolled onto the study. The follow-up period is . 1-year post ASCT.

TABLE 4.3.1: FOLLOW-UP SCHEDULE

Study Visit	Target Day (Day 0 is ASCT)
Baseline	6 weeks prior to transplant
Pre-Conditioning	Within 1 week prior to conditioning*
Transplant	Day 0
HST-NEETs Infusion	Day +3 to +7
Week 2	Day 14 ± **3** days
Week 3	Day 21 ± **3** days
Week 4	Day 28 ± **3** days
Week 5	Day 35 ± **3** days
Week 8 **	Day 56 ± **3** days**
Day 100	Day 100 ± **14** days
Month 6	Day 180 ± **28** days
Month 12	Day 365 ± **28** days

*Can occur locally, does not require a transplant center visit.
**This study visit is only required for participants receiving HST-NEETs outside of the +3 to +7 day window.

4.4. Participant Assessments

Table 4.4 summarizes participant clinical assessments over the course of the study.

4.4.1. Evaluations Prior to Enrollment to Assess Eligibility

The following observation need to be performed within 3 months of enrollment:

1. Hepatitis panel (HbsAb, HbsAg, HbcAb, HCV Ab). If the Hep B core Ab are positive, HepB DNA PCR; if HepC serology is positive, then DNA PCR or NAT Testing.

2. Baseline EKG
3. Baseline metabolic panel: Creatinine clearance (calculated creatinine clearance is permitted per institutional guidelines).
4. Baseline Pulmonary Function Tests: DLCO, FEV1 and FVC.
5. Bone marrow biopsy for pathology is not required, but if performed per institutional standards, results should be submitted.
6. Serum HIV RNA, CD4 Count.
7. Baseline ECHO: Ejection fraction
8. Diagnostic lymphoma pathology specimens (paraffin blocks or 10 unstained slides cut at 5 um thickness and placed on charged glass slides) sent to the Ambinder Laboratory within 3 weeks of ASCT, if specimens are available (See Appendix C).

The following observations need to be performed within 1 month of enrollment:

1. History, physical examination, height and weight, body surface area, neurologic examination, measurement of all palpable peripheral lymph nodes and measurement of other sites of disease present on physical.

   a. Lumbar puncture(s) for determination of presence of CNS disease for non-Hodgkin's lymphoma participants only, is not required, but if performed per institutional standards, results should be submitted.

   b. Duration of AIDS diagnosis, history of prior opportunistic illnesses.

   c. Presence or absence of "B" symptoms (unexplained fevers, night sweats, involuntary weight loss greater than 10% normal body weight).

   d. Medication list to include all antiviral, antibiotics and opportunistic prophylaxis.
2. Pregnancy test for females of childbearing potential, per institutional standards.
3. Karnofsky performance status.
4. CBC with differential, platelet count, creatinine, bilirubin, LDH, ALT, AST.
5. PET-CT or CT: PET-CT or CT scans of neck, chest, abdomen and pelvis. Neck CT only required if previous site of disease.

The following samples should be collected after enrollment and 6-8 weeks prior to transplant at the transplant center:

1. Blood draw for the following study-specific procedures: (see Appendix C)
   a. HST-NEETs Manufacturing.
   b. IPDA (including total HIV proviral DNA).
   c. Single-Copy HIV RNA Assay.
   d. IDM (unless performed by the site within 30 days of HST-NEET peripheral blood collection; see Appendix C)
   e. HLA (unless results available from any prior testing; see Appendix C)

2. CBC with differential, platelet count, creatinine, bilirubin, LDH, ALT, AST. This should be collected at the same time as the IPDA.

The following samples should be collected no more than 1 week prior to initiation of conditioning:

1. Chemistry panel with creatinine, bilirubin, ALT, AST.
2. Plasma DNA (Ig DNA) collected within one week prior to initiation of conditioning. (See Appendix C). **Must be performed at transplant center.**

4.4.2. Post-ASCT Evaluations

1. CBC at least twice a week from Day 0 until ANC greater than 500/mm^3 for 3 days after nadir reached. Thereafter CBC at least once per week until Day 28 (or 4 weeks), then at Day 100, 180 and 365 post-ASCT. CBCs collected at Day 100, 180 and 365 should be drawn at the same time as blood draws for the IPDA.
2. Toxicity assessments at Day 0, 28, 35, 56 (if HST-NEETs cells were received outside the Day 3 to 7 window), 100, 180 and 365 post-ASCT.
3. Disease restaging:
   a. If institutional standard is to perform PET-CT, patient should receive PET-CT at Day 100 post-ASCT. If equivocal results, repeat PET-CT at Day 180.
   b. If institutional standard is to perform CT scan, patient should receive CT scan at Day 100, 180 and 365 post-ASCT.
4. Study-specific blood draw: (see Appendix C)

   a. IPDA (including total HIV proviral DNA) at Day 100, 180 and 365 post-ASCT.

   b. Single-Copy HIV RNA Assay at Day 100, 180 and 365 post-ASCT

   c. Plasma DNA (Ig DNA) assessments at Day 100, 180 and 365 post-ASCT.
5. HIV viral copy number, at Day 100, 180 and 365 post-ASCT by standard clinical assay.

Table 4.4: Study Evaluations

				ASCT	Post-ASCT								
Study Assessments Testing	**Pre-Enrollment**	**6 weeks before conditioning**	**Pre-Conditioning**	**Day 0**	**Day 3 to 7**	**Day 14**	**Day 21**	**Day 28**	**Day 35**	**Day 56[1]**	**Day 100**	**Day 180**	**Day 365**
History and Physical Exam[2]	X					X	X	X	X	X	X	X	X
Karnofsky Performance Score	X		X										
CBC[3]and Chemistries[4]	X	X	X	X	X[4]	X[4]	X[4]	X[4]			X	X	X
Hepatitis Panel (HbsAb HbsAg, HbcAb, HCV Ab) [5]	X												
EKG	X												
CT or PET-CT	X[6]		X[6]								X	X	X
DLCO, FEV1, FVC	X												
Creatinine Clearance[7]	X		X										
Bone marrow biopsy for pathology[8]	X												
HIV RNA, CD4 Count	X										X	X	X
Serology Testing [9]	X												
Ejection Fraction	X												
Pregnancy Test[10]	X												
Diagnostic Lymphoma pathology[11]	X												
Plasma DNA Tumor Monitoring (Ig)			X								X	X	X
Collection for HST-NEETs Manufacturing[12]		X											
IPDA[3,13] & HST-NEETs persistence and expansion		X									X	X	X
Single-Copy HIV-1 RNA Assay[14]		X									X	X	X
HST-NEETs Administration					X								
Toxicity Assessment[15]				X				X	X	X	X	X	X
HIV Titer by standard assay											X	X	X

Footnotes for Table 4.4:

[1] This study visit is only required for participants receiving HST-NEETs outside of the Day +3 to Day +7 window.
[2]To include:
a. Lumbar puncture(s) for determination of presence of CNS disease for non-Hodgkin's lymphoma participants only.
b. Duration of AIDS diagnosis, history of prior opportunistic illnesses.
c. Presence or absence of "B"- symptoms (unexplained fevers, night sweats, involuntary weight loss greater than 10% normal body weight).
d. Medication list to include all antiviral, antibiotics and opportunistic prophylaxis.
This includes Height and Weight, body surface area, neurologic examination, careful measurement of all palpable peripheral lymph nodes and measurement of other sites of disease present on physical.
[3]It is critical that a clinical CBC be collected at the same time as IPDA research sample, as indicated in the table above.
[4]CBC with differential, Platelet Count, Creatinine, Bilirubin, Alkaline Phosphatase, AST, ALT, LDH. To be performed at least twice weekly from Day 0 until ANC greater than 500/mm3 for 3 days after nadir reached. Thereafter, once weekly until Day 28 (or 4 weeks), then at Day 100, 180 and 365 post-ASCT.
[5]If the Hep B Core AB is positive then HepB DNA PCR; if Hep C serology is positive, HCV PCR, or NAT Testing, per institutional standards.
[6]If enrollment has occurred more than 2 months before conditioning starts, a CT scan should be performed to confirm if there has been progression in the interim. CT scans of neck, chest, abdomen and pelvis. Neck CT only required if previous site of disease.
[7]Calculated creatinine clearance is permitted per institutional guidelines.
[8] Bone marrow biopsies for pathology will be collected, optional per institutional standards.
[9]Serology testing will include CMV IgG, HSV-1 and HSV-2 IgG, RPR or VDRL, toxoplasma IgG, VZV IgG, and HTLV-1 antibody, performed per institutional standards.
[10]Pregnancy test is required for females of child-bearing potential and may be performed per institutional practices.
[11]Diagnostic lymphoma pathology local report should be available prior to enrollment. Optional pathology specimens (paraffin block(s) or 10 unstained slides cut at 5 um thickness and placed on charged glass slide) collected pre-enrollment should be sent to the Ambinder Laboratory within 3 weeks of transplant.
[12]100-120 mL whole blood or an 80-100mL apheresis product shipped to CNH at ambient temperature.
[13]Assessment of the Total Proviral HIV DNA will be included in the IPDA sample. A separate blood draw is not required.
[14] Blood for this plasma assay must be centrifuged and plasma frozen within 3 hours of blood draw.
[15] Toxicities related to the BEAM conditioning will be collected at Day 0.

4.4.3. Criteria for Forms Submission

Criteria for timeliness of submission for all study forms are detailed in the BMT CTN 1903 eCRF Completion Guide. Forms that are not entered into Advantage eClinical® within the specified time will be considered delinquent. A missing form will continue to be requested either until the form is entered into the Advantage eClinical® and integrated into the Data Coordinating Center's (DCC) master database or until an exception is granted, as detailed in the Advantage eClinical® User's Guide.

4.4.4. Reporting Participant Deaths

Participant death Information must be entered into Advantage eClinical® within 24 hours of knowledge of the participant's death. If the cause of death is unknown at that time, the cause does not need to be recorded at that time. However, once the cause of death is determined, the form must be updated in Advantage eClinical®.

4.4.5. CIBMTR Data Reporting

The participant must be registered with the CIBMTR and have a valid CRID number. Centers participating in BMT CTN trials must register pre and post-transplant outcomes on all consecutive hematopoietic stem cell transplants done at their institution during their time of participation to the Center for International Blood and Marrow Transplant Research (CIBMTR). Registration is done using procedures and forms of the Stem Cell Transplant Outcomes Database (SCTOD). Enrollment of BMT CTN 1903 must be indicated on the SCTOD pre-transplant registration form. Additionally, CIBMTR pre- and post- transplant Report Forms must also be submitted for all participants enrolled on this trial. CIBMTR forms will be submitted directly to the CIBMTR at the times specified on the Form Submission Schedule.

4.4.6. Protocol Deviation/Violation Reporting

Protocol deviations and violations reporting will be consistent with BMT CTN procedures (BMT CTN Administrative Manual of Procedures, Chapter 5).

4.5. Adverse Event Reporting Requirements

4.5.1. Definitions

Adverse Event: An Adverse Event (AE) is defined as any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease that is temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure.

Expectedness: An adverse event can be Expected or Unexpected

- **Expected adverse events** are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.

- **Unexpected adverse events** are those that vary in nature, intensity or frequency from information in the current adverse event list, the Investigator's Brochure, the package insert, or when it is not included in the informed consent document as a potential risk.

Serious Adverse Event: A serious adverse event (SAE), as defined by per 21 CFR 312.32, is any adverse event that results in one of the following outcomes, regardless of causality and expectedness:

- **Results in death**
- **Is life-threatening.** Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- **Requires or prolongs inpatient hospitalization** (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- **Results in persistent or significant disability/incapacity.** Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- **Is a congenital anomaly or birth defect;** or
- **Is an important medical event** when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Medical and scientific judgment should be exercised in deciding whether expected reporting is also appropriate in situations other than those listed above. For example, important medical events may not be immediately life threatening or result in death or hospitalization but may jeopardize the participant or may require intervention to prevent one of the outcomes listed in the definition above (e.g., suspected transmission of an infectious agent by a medicinal product is considered a Serious Adverse Event). Any event is considered a Serious Adverse Event if it is associated with clinical signs or symptoms judged by the investigator to have a significant clinical impact.

4.5.2. Classification of Adverse Events by Severity

The severity refers to the intensity of the reported event. The Investigator must categorize the severity of each SAE according to the National Cancer Institute (NCI) CTCAE Version 5.0. CTCAE guidelines can be referenced at the following website: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf. For any term that is not specifically listed in the CTCAE scale, intensity will be assigned a grade of one through five using the following CTCAE guidelines:

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

4.5.3. Classification of Adverse Events by Relationship to Investigational Product

The relationship of each reported event to the study therapy will be assessed by the Investigator; after careful consideration of all relevant factors such as (but not limited to) the underlying study indication, coexisting disease, concomitant medication, relevant history, pattern of the SAE, temporal relationship to any study therapy interventions and dechallenge or rechallenge according to the following guidelines:

- **Possibly, Probably, or Definitely Related:** there is a reasonable possibility that the study therapy caused the event. A relationship of possibly, probably or definitely related to the investigational product is considered **related** for the purposes of regulatory authority reporting.
- **Unlikely, or Not Related:** There is no reasonable possibility that the investigational product caused the event. An unlikely or not related relationship to the investigational product is considered **not related** for the purposes of regulatory authority reporting.

4.5.4. BMT CTN Adverse Event Reporting Guidelines

Adverse event reporting will be consistent with BMT CTN procedures (BMT CTN Administrative Manual of Procedures, Chapter 6). It is BMT CTN policy that Aes must be reported irrespective of the attribution of the event by the investigator to the study intervention. Determination of expectedness for events will be at the discretion of the investigator.

The BMT CTN 1903 protocol has two distinct time periods of study intervention: Segment 0 and Segment A. Segment 0 includes a study intervention of peripheral blood or apheresis collection for HST-NEET generation, a period of rest from study intervention, and then the beginning of the protocol defined conditioning regimen. Segment A begins at transplant Day 0 and includes infusion of the HST-NEET product several days later. Surrounding study interventions (around the time of Segment 0 interventions, and from the start of Segment A through the study follow up period of one year), all unexpected SAEs will be reported. If a participant does not receive the HST-NEET product (due to manufacturing difficulties, etc.), unexpected SAE collection would then stop at the time of knowledge that the participant will not receive the product. No further expedited SAE reporting would be required from that time forward.

Unexpected, serious adverse events (SAEs) will be reported through an expedited AE reporting system via Advantage eClinical®. **Unexpected, life-threatening and fatal SAEs must be**

reported within 24 hours of knowledge of the event. All other unexpected SAEs must be reported within 3 business days of knowledge of the event. Events entered in Advantage eClinical® will be reported using NCI's Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0.

Expected Aes will be reported using NCI's Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 at regular intervals as defined on the Form Submission Schedule, including calendar-driven case report forms (e.g., Toxicity and GVHD) or event-driven case report forms (e.g., Relapse/Progression, Infection, and Death). **Any expected life-threatening SAE not collected on another study form must be reported through the expedited AE reporting system via Advantage eClinical®.**

The Data and Safety Monitoring Board (DSMB) will receive reports of all unexpected SAEs, pregnancies, and HST-NEET infusion related reactions that meet SAE criteria upon review by the BMT CTN Medical Monitor. Summary reports for all reported SAEs will be reviewed by the DSMB on a semi-annual basis.

4.5.5. Adverse Events of Special Interest

Adverse events of special interest (AESIs) are required to be reported by the investigator within 24 hours of notification of the event.

Adverse events of special interest for this study are as follows:

- Infusion related reaction including allergic reactions and anaphylaxis occurring during or post HST-NEETs infusion (infusion reactions occurring within 24 hours of the infusion).

4.5.6. Procedure in Case of Pregnancy

If a female participant becomes pregnant during the study dosing period or within 90 days from the HST-NEETs infusion, the investigator should report within 24 hours through an expedited AE reporting system via Advantage eClinical®. The expected date of delivery or expected date of the end of the pregnancy, last menstruation, estimated conception date, pregnancy result, neonatal data and other related information will be requested.

The investigator will follow the medical status of the mother, as well as the fetus, as if the pregnancy is an SAE and will report the outcome. Additional information regarding the outcome of a pregnancy (which is categorized as an SAE) is mentioned below.

- "Spontaneous abortion" includes miscarriage, abortion and missed abortion
- Death of an infant within 1 month after birth should be reported as an SAE regardless of its relationship with the study drug
- If an infant dies more than 1 month after the birth, it should be reported if a relationship between the death and intrauterine exposure to the study drug is judged as "possible" by the investigator
- In the case of a delivery of a living newborn, the "normality" of the infant is evaluated at the birth

- Unless a congenital anomaly is identified prior to spontaneous abortion or miscarriage, the embryo or fetus should be assessed for congenital defects by visual examination

Information will be collected at the time of delivery/birth and 6 months and 12 months after birth. If the participant completes the final study follow-up prior to 12 months after birth, a final status should be reported following the final study visit.

CHAPTER 5

5. STATISTICAL CONSIDERATIONS

5.1. Study Overview

This is a Phase II single arm multicenter trial designed to evaluate the feasibility and efficacy of ASCT followed by administration of HST-NEETs for treatment of HIV associated lymphoma. The study has two primary objectives: (1) estimate the HST-NEETs infusion proportion within 1 week of ASCT and (2) test for a reduction in the intact proviral reservoir using the intact proviral DNA assay (IPDA)at 6 months post-HSCT. The target enrollment is 12 participants.

5.1.1. Accrual

The study will accrue 12 participants. It is estimated that four years of accrual will be necessary to enroll the targeted sample size.

5.1.2. Primary Endpoints

There are two primary endpoints for this study. The first primary endpoint is the infusion of HST-NEETs within 1 week of ASCT. The second primary endpoint is the reduction in intact proviruses (measured with IPDA in intact proviruses per million CD4 cells) at 6 months post-HSCT.

5.1.3. Primary Hypotheses

HST-NEET infusion proportion
The study is not formally powered to test the HST-NEET infusion proportion. Instead, it is designed to estimate this rate.

Intact provirus reduction in participants who were transfused
The null hypothesis is that there is zero difference between the mean baseline and post-intervention logarithm of IPDA vs. the alternative that the mean baseline and post-intervention logarithm of IPDA difference is not zero.

5.2. Sample Size Calculations

Since this is a feasibility pilot study, the sample size of 12 participants is based on the anticipated enrollment over a 4-year accrual period. Based on enrolling 12 participants, we provide the possible confidence intervals for the HST-NEET infusion proportion (Table 5.2.1A) and the statistical power (Table 5.2.1B) for the IPDA outcome. Participants will only count towards the study sample size if they had their sample collected and submitted for the HST-NEETs manufacturing.

HST-NEET infusion proportion:

Table 5.2.1A presents the exact 90% confidence intervals corresponding to zero to 12 infusions of HST-NEETs.

Table 5.2.1A. Exact 90% confidence intervals for the possible HST-NEET infusion proportions in the 12 participants

Number of HST-NEET Infusions	Infusion Proportion (%)	Exact (Clopper-Pearson) 90% Confidence Interval	Number of HST-NEET Infusions	Infusion Proportion (%)	Exact (Clopper-Pearson) 90% Confidence Interval
0	0.00	(0.00, 22.09)	7	58.33	(31.52, 81.90)
1	8.33	(0.43, 33.87)	8	66.67	(39.09, 87.71)
2	16.67	(3.05, 43.81)	9	75.00	(47.27, 92.81)
3	25.00	(7.19, 52.73)	10	83.33	(56.19, 96.95)
4	33.33	(12.29, 60.91)	11	91.67	(66.13, 99.57)
5	41.67	(18.10, 68.48)	12	100	(78.91, 100)
6	50.00	(24.53, 75.47)			

Intact Provirus reduction in participants who were transfused:
Pilot estimates for the baseline distribution of the natural logarithm of IPDA indicate a standard deviation of 1.46. We assume the correlation between baseline and post-intervention log (IPDA) measurements is 0.5. Using a paired t-test with a two-sided significance level of 10%, we estimate that 12 infused participants will have 84.8% statistical power to detect a mean difference of -1.2040 between the baseline and post-intervention log (IPDA). The mean baseline and post-intervention log (IPDA) difference of -1.2040 corresponds to a reduction of 70% (100%[1 – exp(-1.2040)]) in IPDA on the original scale. Participants who were not infused will not be included in this analysis.

Table 5.2.1B Power Analysis to Detect the Reduction of Intact Proviruses on the Original Scale

		No. of Infused Participants			
	Mean Percent Reduction of IPDA	6	8	10	12
Statistical Power (%)	75%	63.9	77.9	86.9	92.4
	70%	53.8	67.5	77.6	84.8
	60%	37.8	48.3	57.5	65.3
	40%	19.2	23.2	27.1	30.9
	20%	11.8	12.6	13.4	14.2

5.3. Interim Analysis and Stopping Guidelines

5.3.1. Interim Analysis

There will be no interim analyses for efficacy or futility.

5.3.2. Safety Monitoring

Monitoring of safety will be conducted by reviewing HST-NEET treatment-related mortality (TRM) within 30 days of transplantation and infusion-related toxicities. Any HST-NEET treatment-related mortality event observed within 30 days of transplantation will undergo a

thorough safety review by the NHLBI DSMB prior to treatment of subsequent participants. Policies and composition of the DSMB are described in the BMT CTN's Manual of Procedures. As there is underlying risk of TRM associated with auto-transplantation, Table 5.3.1 shows the probability of observing 1 or more, 2 or more, and 3 or more events as a function of the underlying TRM rate to help guide decision making during the safety review. The probability of observing three or more events is 0.02 and 0.75 if the TRM rate is 5% and 30%, respectively. If the TRM rate is 5%, the probability of observing one or more events, two or more events and three or more events is 0.46, 0.12 and 0.02, respectively.

Table 5.3.1. Probabilities of Observing Events Based On Underlying Day 30 TRM Rates

Day 30 TRM Rate	5%	10%	20%	25%	30%
Probability of 1 or more events	0.46	0.72	0.93	0.97	0.99
Probability of 2 or more events	0.12	0.34	0.73	0.84	0.91
Probability of 3 or more events	0.02	0.11	0.44	0.61	0.75

The frequency of Grade 3 or higher infusion-related toxicities with a duration of 24 hours or more will also be monitored. If three or more of these events are observed, the NHLBI will be notified and the DSMB will be consulted regarding continuation of the study. This stopping guideline serves as a trigger for consultation with the DSMB for additional review and is not a formal stopping rule that would mandate closure of study enrollment.

In the event of graft failure, following HST-NEET infusion, the protocol will be temporarily halted and reviewed by the NHLBI DSMB. Graft failure will be defined as a failure to achieve three consecutive labs of greater than or equal to 500 neutrophils/µL by Day 100.

5.4. Demographic and Baseline Characteristics

Demographics and baseline characteristics will be summarized for all participants using descriptive statistics. Characteristics to be examined are age, gender, race/ethnicity, performance status, disease stage, genotype, HIV viral load, CD4+ counts, and number of prior chemotherapy regimens as treatment of primary malignancy and number of prior HIV regimens.

5.5. Analysis Plan

Analysis populations include:

- Feasibility Population
  - All participants who have the sample collected and submitted for HST-NEETs manufacturing.
- Transplant Population
  - All participants enrolled on the study who proceed to transplant.
- Infusion Population
  - All participants who are enrolled on the study that received both a transplant and HST-NEETs.

5.5.1. Analysis of the Primary Endpoints

HST-NEET infusion proportion:
We will report the proportion of HST-NEET infusion. Additionally, a 90% Clopper-Pearson confidence interval approach for that rate will be computed.[47] This analysis will include all 12 participants.

Intact provirus reduction in participants who were transfused:
The difference in logarithm of IPDA measured pre-transplant and at 6 months post-transplant will be compared using a paired t-test. The difference in means will be computed with 90% confidence interval based on the t-distribution along with the corresponding p-value. This analysis will be done for all infused participants.

5.5.2. Analysis of Secondary Endpoints

Progression-Free Survival (PFS)
The event is relapse/progression or death or receive anti-lymphoma therapy, other than post-transplant consolidative localized radiation (maximum 3 sites) to sites of prior bulk disease pre-transplant (greater than 3cm). The time to this event is the time from transplant until death, relapse/progression, receipt of anti-lymphoma therapy, or last follow up, whichever comes first. Progression-free survival (PFS) will be estimated using the Kaplan-Meier product limit estimator and Kaplan-Meier plots with 95% confidence intervals using Greenwood's formula and the log-log transformation. The PFS probability and 95% confidence interval will be calculated at 6 months and one-year post-transplant.

Acute Infusion Related Toxicities
Toxicities that occur over the course of time will be tabulated using the version 5.0 CTCAE criteria. The clinical safety monitoring period for HST-NEETs will be 28 days following infusion. The proportion of participants developing toxicity will be described by type of toxicity, grade, and time period. The 95% Clopper-Pearson confidence interval will be used. This analysis will be done on the infusion or transplant population.

Intact Proviral Reservoir at One Year
Impact on intact proviral reservoir will be assessed using the IPDA at 12 months following autologous HCT. The difference in log (IPDA) measured pre-transplant and at 12 months post-transplant will be compared using a paired t-test. The difference in means will be computed with 95% confidence interval based on the t-distribution along the corresponding p-value. This analysis will be done on the infusion population.

5.5.3. Analysis of Exploratory Endpoints

CR and CR+PR Rate at Day 100 after ASCT
The frequencies and proportions of participants who have a CR (or PR) will be described with 95% Clopper-Pearson confidence intervals at Day 100 after ASCT. This analysis will be done on the transplant population.

Overall Survival
The event is death by any cause. The time to this event is from transplant. Participants are censored at the time of last follow-up. We will estimate the Kaplan Meier curve at 6-month and 1-year overall survival (OS) probability based on the Kaplan-Meier product limit estimator with corresponding 95% confidence intervals using Greenwood's formula and the log-log transformation.

Time to Hematopoietic Recovery
Hematologic function will be defined by ANC greater than 500, Hemoglobin greater than 10g/dL without transfusion support, and platelets greater than 100,000 and measured at Day 100 and . 1-year. Use of growth factors will be noted. Time to neutrophil recovery and platelet engraftment from transplant will be estimated using cumulative incidence function with corresponding 95% confidence interval with death prior to engraftment as the competing risk.

Incidence of Infections
The incidence rate of infections is defined by the number of infections divided by the total person-time accumulated over the duration of the study. All Grade 2 and higher infections will be reported according to the BMT CTN technical guidelines from Day 0 up to 1-year post-transplant. The incidence rate of infections will be computed with corresponding 95% exact confidence interval based on the Poisson distribution.

Non-Relapse Mortality
Non-Relapse Mortality (NRM) is defined as death occurring in a participant without relapse or progression. Progression is a competing risk event. A cumulative incidence curve will be computed along with a 95% confidence interval at 6 months and at 1-year post-transplant.

Toxicity:
Toxicities related to the BEAM conditioning regimen and HST-NEETs infusion beyond the 24-hour acute toxicity monitoring period will be defined by using the version 5.0 CTCAE criteria. The data will be collected up to day +28. All grade of toxicity related to HST-NEETs will be collected. Only grade 3 or higher conditioning regimen related toxicities will be collected. BEAM conditioning regimen toxicity data will still be collected through Day 28 for participants that do not receive the HST-NEET product. The frequencies and proportions of participants with toxicities will be described with 95% Clopper-Pearson confidence intervals.

Ig DNA in Blood
Blood specimens will be collected within 1 week prior to the initiation of conditioning, and at Day 100, 6 months, and 1-year post-transplant. The presence of clonal Ig DNA in plasma will be assessed at each time point and will be summarized using descriptive statistics with plots of the outcome vs Days. We will model the Ig DNA in blood using a mixed model with Days as a covariate.

Intact Proviral Reservoir at Day 100
Impact on intact proviral reservoir will be assessed using the IPDA at Day 100 following autologous HCT. The difference in log (IPDA) measured pre-transplant and at Day 100 post-transplant will be compared using a paired t-test. The difference in means will be computed with

95% confidence interval based on the t-distribution along the corresponding p-value. This analysis will be done on the infusion population.

HIV RNA in Blood

The difference in log (HIV RNA) measured pre-transplant and at 6 months post-transplant will be compared using a paired t-test. The difference in means will be computed with 95% confidence interval based on the t-distribution along with the corresponding p-value. This analysis will be done for all participants whose HIV RNA could be ascertained. Additionally, HIV RNA will be summarized using descriptive statistics with plots of the outcome vs Days. We will model the HIV RNA in blood using a mixed model with Days as a covariate.

Total Proviral HIV DNA (TPDNA)

Impact on total proviral HIV DNA will be assessed using the TPDNA at 6 months following autologous HCT. The difference in log (TPDNA) measured pre-transplant and at 6 months post-transplant will be compared using a paired t-test. The difference in means will be computed with 95% confidence interval based on the t-distribution along the corresponding p-value. This analysis will be done on the infusion population.

Persistence and expansion of HST-NEETs in vivo

Persistence and expansion of HST-NEETs will be measured by frequency of HIV-1 antigen-specific (gag, pol, nef) CD8+ T-cells by ELIspot at baseline and post-infusion at timepoints at Day 100, 6 months, and 1-year post transplant. Change in T cell responses from baseline to post-infusion, measured by frequency of cells secreting IFN-γ by multimer analysis and/or intracellular cytokine staining and/or ELIspot and/or TCR sequencing will be done depending on PBMC cell numbers available and reagent availability.

Relapse/Progression of Primary Disease

Relapse/progression will be summarized using a cumulative incidence curve along with a 95% confidence interval at 6 months and at 1-year post-transplant. Initiation of anti-lymphoma therapy will also be considered an event for relapse/progression. Death in the absence of relapse/progression will be considered a competing risk.

These will be summarized at each time point using descriptive statistics with plots of the outcome vs Days. We will model these outcomes using a mixed model with Days as a covariate.

APPENDIX A: HUMAN SUBJECTS

APPENDIX A

HUMAN SUBJECTS

1. Subject Consent

Candidates for the study will be identified as described in Chapter 4 of the protocol. The Principal Investigator or his/her designee at each transplant center will contact the candidates, provide them with information about the purpose of the study and obtain voluntary consent if the candidates agree to participate. The BMT CTN will provide a template of the consent form to each center. Each center will add their NMDP IRB-approved boiler-plate language to the consent and submit it for review by the NMDP Institutional Review Board (IRB). The DCC will verify the adequacy of the consent forms prior to submission to the IRB. The NMDP IRB will provide evidence of IRB approval.

2. Confidentiality

Confidentiality will be maintained by individual names being masked and assigned a participant identifier code. The code relaying the participant's identity with the ID code will be kept separately at the center. The ID code will be transmitted to the BMT CTN Data Coordinating Center upon enrollment.

3. Participation of Women and Minorities and Other Populations

Women and ethnic minorities and other populations will be included in this study. Accrual of women and minorities at each center will be monitored to determine whether their rates of enrollment are reflective of the distribution of potentially eligible women and minorities expected from data reported to the CIBMTR and from published data on incidence of DLCL in these groups. Centers will be notified if their rates differ significantly from those expected and asked to develop appropriate recruitment reports.

4. GCP

This study will be conducted in accordance with standards of Good Clinical Practice (as defined by the International Council for Harmonization) and all applicable national and local regulations.

APPENDIX B: LUGANO CLASSIFICATION

The following tables are derived from Lymphoma Response to Immunomodulatory Therapy Criteria[46]

	PET-CT Based Response	**CT-Based Response**
Complete Response (CR)	**Complete metabolic response (CMR)**	**Complete radiologic response (CR) (all of the following)**
Lymph nodes and extra lymphatic sites	Score 1, 2, or 3[a] with or without a residual mass on 5PS† It is recognized that in Waldeyer's ring or extranodal sites with high physiologic uptake or with activation within spleen or marrow (*e.g.,* with chemotherapy or myeloid colony-stimulating factors), uptake may be greater than normal mediastinum and/or liver. In this circumstance, complete metabolic response may be inferred if uptake at sites of initial involvement is no greater than surrounding normal tissue even if the tissue has high physiologic uptake	Target nodes/nodal masses must regress to less than or equal to 1.5 cm in Ldi No extra lymphatic sites of disease
Non-measured lesion	Not applicable	Absent
Organ enlargement	Not applicable	Regress to normal
New lesions	None	None
Bone marrow	No evidence of FDG-avid disease in marrow	Normal by morphology; if indeterminate, IHC negative
Partial Response (PR)	**Partial metabolic response (PMR)**	**Partial remission (PR) (all of the following)**
Lymph nodes and extra lymphatic sites	Score 4 or 5† with reduced uptake compared with baseline and residual mass(es) of any size At interim, these findings suggest responding disease At end of treatment, these findings indicate residual disease	Greater than or equal to 50% decrease in SPD of up to 6 target measurable nodes and extranodal sites When a lesion is too small to measure on CT, assign 5 mm X 5 mm as the default value When no longer visible, 0 X 0 mm For a node > 5 mm X 5 mm, but

	PET-CT Based Response	CT-Based Response
		smaller than normal, use actual measurement for calculation
Non-measured lesions	Not applicable	Absent/normal, regressed, but no increase
Organ enlargement	Not applicable	Spleen must have regressed by greater than 50% in length beyond normal
New lesions	None	None
Bone marrow	Residual uptake higher than uptake in normal marrow but reduced compared with baseline (diffuse uptake compatible with reactive changes from chemotherapy allowed). If there are persistent focal changes in the marrow in the context of a nodal response, consideration should be given to further evaluation with MRI or biopsy or an interval scan	Not Applicable
No Response or Stable Disease	**No metabolic response (NMR)**	**Stable disease (SD)**
Target nodes/nodal masses, extranodal lesions	Score 4 or 5 with no significant change in FDG uptake from baseline at interim or end of treatment	Less than 50% decrease from baseline in SPD of up to 6 dominant, measurable nodes and extranodal sites; no criteria for progressive disease are met
Non-measured lesions	Not applicable	No increase consistent with progression
Organ enlargement	Not applicable	No increase consistent with progression
New lesions	None	None
Bone marrow	No change from baseline	Not Applicable

	PET-CT Based Response	CT-Based Response
Progressive disease (PD)	***** Excludes patients who meet criteria for Indeterminate Response (IR) *****	
	Progressive metabolic disease (PMD)	**Progressive disease (PD) requires at least 1 of the following**
Individual target nodes/nodal masses Extranodal lesions	Score 4 or 5 with an increase in intensity of uptake from baseline and/or New FDG-avid foci consistent with lymphoma at interim or end-of-treatment assessment	An individual node/lesion must be abnormal with: Ldi greater than 1.5 cm and Increase by greater than or equal to 50% from PPD nadir and An increase in Ldi or Sdi from nadir 0.5 cm for lesions less than or equal to2 cm 1.0 cm for lesions greater than 2 cm In the setting of splenomegaly, the splenic length must increase by >50% of the extent of its prior increase beyond baseline (*e.g.*, a 15 cm spleen must increase to greater than16 cm). If no prior splenomegaly, must increase by at least 2 cm from baseline New or recurrent splenomegaly

	PET-CT Based Response	CT-Based Response
Non-measured lesions	None	New or clear progression of preexisting non-measured lesions
New lesions	New FDG-avid foci consistent with lymphoma rather than another etiology (*e.g.*, infection, inflammation). If uncertain regarding etiology of new lesions, biopsy or interval scan may be considered	Regrowth of previously resolved lesions A new node greater than 1.5 cm in any axis A new extranodal site greater than 1.0 cm in any axis; if less than 1.0 cm in any axis, its presence must be unequivocal and must be attributable to lymphoma Assessable disease of any size unequivocally attributable to lymphoma
Bone marrow	New or recurrent FDG-avid foci	New or recurrent involvement

Abbreviations: 5PS, 5-point scale; CT, computed tomography; FDG, fluorodeoxyglucose; IHC, immunohistochemistry; Ldi, longest transverse diameter of a lesion; MRI, magnetic resonance imaging; PET, positron emission tomography; PPD, cross product of the Ldi and perpendicular diameter; Sdi, shortest axis perpendicular to the Ldi; SPD, sum of the product of the perpendicular diameters for multiple lesions.

100. A score of 3 in many patients indicates a good prognosis with standard treatment, especially if at the time of an interim scan. However, in trials involving PET where de-escalation is investigated, it may be preferable to consider a score of 3 as inadequate response (to avoid under treatment). Measured dominant lesions: Up to six of the largest dominant nodes, nodal masses, and extranodal lesions selected to be clearly measurable in two diameters. Nodes should preferably be from disparate regions of the body and should include, where applicable, mediastinal and retroperitoneal areas. Non-nodal lesions include those in solid organs (*e.g.*, liver, spleen, kidneys, and lungs), GI involvement, cutaneous lesions, or those noted on palpation.

Non-measured lesions: Any disease not selected as measured, dominant disease and truly assessable disease should be considered not measured. These sites include any nodes, nodal masses, and extranodal sites not selected as dominant or measurable or that do not meet the requirements for measurability but are still considered abnormal, as well as truly assessable disease, which is any site of suspected disease that would be difficult to follow quantitatively with measurement, including pleural effusions, ascites, bone lesions, leptomeningeal disease, abdominal masses, and other lesions that cannot be confirmed and followed by imaging. In Waldeyer's ring or in extranodal sites (*e.g.*, GI tract, liver, bone marrow), FDG uptake may be greater than in the mediastinum with complete metabolic response but should be no higher than surrounding normal physiologic uptake (*e.g.*, with marrow activation as a result of chemotherapy or myeloid growth factors).

† PET 5PS: 1, no uptake above background; 2, uptake less than or equal to mediastinum; 3, uptake greater than mediastinum but less than or equal to liver; 4, uptake moderately greater than liver; 5, uptake markedly higher than liver and/or new lesions; X, new areas of uptake unlikely to be related to lymphoma.

APPENDIX C: LABORATORY PROCEDURES

APPENDIX C

LABORATORY PROCEDURES

1. HST-NEETS PRODUCT MANUFACTURING, HLA TYPING, AND INFECTIOUS DISEASE MARKER (IDM) TESTING

Peripheral Blood Collection:
100-120 mL of peripheral blood is collected in multiple 10 mL-fill sodium heparin tubes for HST-NEETs product manufacturing within 6-8 weeks of transplant.
Apheresis:
80-100mL of MNCs with $1x10^9$ TNC, with >90% viability collected via non-mobilized leukapheresis for HST-NEETs product manufacturing within 6-8 weeks of transplant. If the sample is does not meet the viability the sample should not be submitted.

Collect an additional 20-24 mL of peripheral blood for testing. For Infectious Disease testing collect 2 purple EDTA and 2 red Vacutainer tubes (4mL per tube). See specific IDM tests listed in the table below. Collect 4-8 mL in a yellow top ACD tube for HLA testing. HLA testing is performed before and after manufacturing to verify product identity. The samples are shipped by priority overnight FedEx to the Cellular Therapy Laboratory at Children's National Hospital.

2. INTACT PROVIRAL DNA ASSAY (IPDA) AND HST-NEETS PERSISTENCE AND EXPANSION

There is a critical need for accurate, HIV reservoir assays to evaluate potential HIV cure approaches. In this correlative study, we will be employing the recently developed intact proviral DNA assay (IPDA). This assay takes advantage of advancements in droplet digital PCR, where a single HIV genome can be amplified by PCR within an oil droplet. Two amplicons are used, which can with high sensitivity distinguish between defective and intact proviral HIV genomes. Note we will also extract total HIV DNA data from the IPDA assay, even if sequence diversity causes the IPDA to fail. Published Env primers/probe and the backup Env primers/probe that are reported in a Biorxiv manuscript will also be reported for all donors. With both of these together, the assays should cover most of the inter-individual diversity. It also gives us an opportunity to flag and further investigate cases where within-donor HIV diversity causes the IPDA to underestimate the reservoir[48].

Persistence of HST-NEETs will be measured by frequency of HIV-1 antigen-specific (gag, pol, nef) CD8+ T-cells by ELIspot at baseline and post-infusion at timepoints at Days 100, 180 and 365 post-transplant. Change in T cell responses from baseline to post-infusion, measured by frequency of cells secreting IFN-γ by multimer analysis and/or intracellular cytokine staining and/or ELIspot and/or TCR sequencing will be done depending on PBMC cell numbers available and reagent availability.

70 mL of peripheral blood is collected in multiple 10 mL-fill sodium heparin tubes at approximately 4-8 weeks prior to transplant and at Days 100, 180 and 365 post-transplant. The blood tubes are shipped by priority overnight FedEx to the Cellular Therapy Laboratory at Children's National Hospital.

3. CHARACTERIZATION OF THE UTILITY OF PLASMA DNA MARKERS FOR AIDS LYMPHOMA

Tumor DNA detected in plasma is emerging as a potentially useful tumor marker. Clonal Ig DNA tumor marker, as recognized by Ig rearrangements or characteristic somatic hypermutation in participants with HIV associated lymphomas will be evaluated in this correlative laboratory study. In previous studies, clonal Ig DNA is characteristic of a particular participant's B cell tumor and is detected in approximately half of AIDS diffuse large B cell lymphoma prior to treatment. In those studies, participants in whom clonal Ig DNA persists in plasma inevitably relapse. Plasma DNA (clonal Ig) will be measured prior to conditioning, prior to stem cell infusion, and at Day +100, 6 months and 1-year post-transplant will be summarized using descriptive statistics.

25 mL of peripheral blood is collected in three 10-mL fill STRECK Cell Free BCT tubes within 1 week prior to initiation of ablative therapy, and on Days 100, 180 and 365 post-transplant. The blood tubes are shipped at ambient temperature on the day of collection by priority overnight FedEx to the Ambinder Laboratory at Johns Hopkins University.

4. CIRCULATING PLASMA HIV RNA MEASUREMENTS

In the current study, we will monitor the presence of circulating HIV RNA before and after transplant and following the administration of HST-NEETs. HIV viral load will be measured with a sensitive investigational single copy PCR assay (SCA) with a viral RNA detection limit of 0.38 copy/ml. In patients with no detectable viral load by standard clinical assays, this assay regularly detects low copy number viral RNA. This RNA is believed to be released from latency compartments established in hematopoietic cells.

5 mL of peripheral blood is collected in a single EDTA Vacutainer tube approximately 4-8 weeks prior to transplant and at Days 100, 180 and 365 post-transplant. The samples will be promptly processed locally within 3 hours of collection and plasma aliquots frozen at -70° C. The frozen plasma aliquots will be periodically batch-shipped by priority overnight FedEx to the Mellors Laboratory at the University of Pittsburg School of Medicine.

Research Sample Collection Schedule for Participant Blood Samples for Required Product Manufacturing and Protocol-Defined Research Testing

RESEARCH TOPIC	RESEARCH SAMPLE	TYPE OF SAMPLE	SAMPLE COLLECTION, PROCESSING AND STORAGE REQUIREMENTS	SAMPLE COLLECTION TIME POINTS	SHIPPING SPECIFICATIONS
HST-NEETs Product Manufacturing, HLA typing, **Infectious Disease Marker (IDM) Testing**[1]	**Peripheral Blood or apheresis product for HST-NEETs Manufacturing, HLA typing, and IDM Testing**	**HST-NEETs**[2] (ALC ≥ 800/uL): 100-120 mL peripheral blood collected in multiple 10 mL sodium heparin, **HST-NEETs**[2] (ALC ≥ 400/uL and < 800/uL): MNCs collected via apheresis, **IDM:** 16mL peripheral blood collected in 2 purple EDTA and 2 red Vacutainer tubes (4mL per tube) **HLA:** 7-10mL peripheral blood collected in 1 yellow ACD tube	Peripheral Blood: Gently mix blood tubes with the anticoagulant by inverting the tube 8-10 times. Apheresis: Collected using either the Optia or Amicus device. Store at room temperature while preparing to ship to manufacturing laboratory	Approximately 6-8 weeks prior to transplant	Cellular Therapy Laboratory at Children's National Hospital

[1] **IDM testing includes: Chagas Disease (if applicable), Hepatitis B Surface Antigen, Hepatitis B Core, Hepatitis C Core, HIV 1 and 2, TAQ Screen MPX (HBV, HCV, HIV-1 group M RNA, HIV-1 Group O), NAT HCV, NAT HIV, NAT HBV, NAT West Nile Virus, Serological Test for Syphilis, CMV IgG/IgM, Anti-HTLV I/II. IDM testing may need to be repeated if the HST-NEET peripheral blood is recollected.**

[2] **HST-NEETs:** The manufacturing materials for HST-NEETs can be collected 1 of 2 ways. If the participant has a ALC above or equal to 800/uL the participant will have their manufacturing sample collected via blood draw (100-120mL). If the participant has an ALC lower than 800 ALC and ≥ 400, the manufacturing sample will be collected via apheresis. If a repeat manufacturing sample is needed the sample will be collected via apheresis.

Required Research Samples					
RESEARCH TOPIC	**RESEARCH SAMPLE**	**TYPE OF SAMPLE**	**SAMPLE COLLECTION, PROCESSING AND STORAGE REQUIREMENTS**	**SAMPLE COLLECTION TIME POINTS**	**SHIPPING SPECIFICATIONS**
Intact Proviral DNA Assay (IPDA) and HST-NEETs Persistence and Expansion	**Peripheral Blood for IPDA Measurements and HST-NEETs Evaluation**	70 mL peripheral blood collected in seven 10 mL sodium heparin Vacutainer tubes	Gently mix blood tubes with the anticoagulant by inverting the tube 8-10 times. Store at room temperature while preparing to ship to manufacturing laboratory	**Pre-transplant** Approximately 6-8 weeks prior to transplant **Post-transplant** Days 100, 180, 365	Peripheral blood tubes will be shipped at ambient temperature on the day of collection, to the Cellular Therapy Laboratory at Children's National Hospital by priority overnight FED EX delivery for processing and research testing. Guidelines for specimen handling and shipment to the Cellular Therapy Laboratory is detailed in the BMT CTN 1903 Laboratory Sample Guide.
Plasma DNA Tumor Monitoring	**Clonal Ig B-cell DNA**	25 mL peripheral blood sample collected in three 10 mL Streck Cell-Free DNA BCT® tubes.	Gently mix blood with cell stabilizer by inverting the tube 8-10 times. Store at room temperature while preparing to ship to project laboratory.	**Pre-transplant** Within 1 weeks prior to initiation of ablative therapy. **Post-transplant** Days 100, 180, 365	Peripheral blood tubes will be shipped at ambient temperature on the day of collection, to the Ambinder Laboratory at Johns Hopkins University by priority overnight FED EX delivery for processing and research testing. Guidelines for specimen handling and shipment to the Ambinder Laboratory is detailed in the BMT CTN 1903 Laboratory Sample Guide.
Lymphoma Pathology	**Diagnostic Tissue** **(If Available)**	Paraffin block(s) or 10 unstained slides cut at 5 um thickness and placed on charged glass slides	Store at room temperature while preparing to ship to project laboratory.	Specimens on enrolled participants will be submitted to project lab within 3 weeks of transplant.	Tissue samples will be shipped at ambient temperature to the Guidelines for specimen handling and shipment to the Ambinder Laboratory at Johns Hopkins University is detailed in the BMT CTN 1903 Laboratory Sample Guide.
Plasma HIV Measurement	**Single-Copy HIV RNA Assay**	5 mL peripheral blood collected in an EDTA containing Vacutainer tube	Gently mix blood tubes with the anticoagulant by inverting the tube 8-10 times. Blood samples will need to be centrifuged, and plasma separated, aliquoted into cryovials and frozen at -70° C within 3 hours of blood collection. Detailed sample processing information will be found in the BMT CTN 1903 Laboratory Sample Guide.	**Pre-transplant** Approximately 6-8 weeks prior to transplant **Post-transplant** Days 100, 180, 365	Frozen plasma sample aliquots will be periodically batched-shipped to the Mellors Laboratory at the University of Pittsburgh School of Medicine by priority overnight FedEx® delivery. Guidelines for specimen handling and shipment to the Mellors Laboratory is detailed in the BMT CTN 1903 Laboratory Sample Guide.

APPENDIX D: SUGGESTED PROPHYLAXIS

HIV MANAGEMENT

ANTIRETROVIRALS AND BILIRUBIN

The following antiretroviral medications lead to elevated bilirubin in the absence of other evidence of liver dysfunction.

APPENDIX D

SUGGESTED PROPHYLAXIS

Infectious prophylaxis for HIV patients undergoing HCT will include prophylaxis for:

1. Bacteria: In keeping with the BMT CTN MOP and local institutional standards.
2. Pneumocystis Jiroveci Pneumonia: Prophylaxis will be administered until CD4 counts are greater than 200 and for a minimum of 6 months. Several effective regimens are available. Patient tolerance (nausea, allergic reaction, G6PD or other considerations) may contraindicate a particular regimen. Choices in order of preference are 1) TMP/SMX 1 DS daily, 2) TMP/SMX 1 SS daily, 3) Atovaquone 1500 mg daily, 4) Dapsone 100 mg daily, 5) Aerosolized pentamidine monthly.
3. Toxoplasmosis: Patients on TMP/SMX do not require additional prophylaxis. If toxoplasma IgG is positive and TMP/SMX cannot be used, then patients should be prophylaxed for at least 3 months after HCT and until CD4 greater than 100. This prophylaxis may be either: 1) atovaquone 1500 mg po daily or 2) dapsone 50 mg po daily and pyrimethamine 50 mg/week and leucovorin 25 mg/week
4. Fungi: Anti-fungal prophylaxis will be per local institutional practice. It is noted that in histoplasma endemic areas (Midwest and Puerto Rico) antifungal prophylaxis is standard for CD4 less than 150 and would be appropriate for at least 3 months after HCT and until CD4 greater than 150.
5. HSV/VZV: One of the following regimens should be used for. 1-year after HCT i.e., Acyclovir 400 - 800 mg bid, valaciclovir 500 mg bid, or famciclovir 500 mg po bid.
6. Hepatitis:
    a. Patients with positive hepatitis B surface antigen should be evaluated for viral DNA replication (viral load) by a quantitative PCR method before enrolling the patient on the study.
    b. Newer generation of anti-hepatitis B agents, like Tenofovir, should be started in those with detectable Hepatitis B viral load according to institutional preferences. The goal of the treatment should be achieving undetectable (less than 500 copies/ml) viral load status before stem cell mobilization chemotherapy.
    c. Patients should be maintained on anti-Hepatitis B treatment throughout the transplant and at least 12 months after the transplant.
    d. Patients with hepatitis-C infection may be enrolled on the trial providing the above Hepatic criteria are met. Anti-hepatitis C treatment with directly acting antivirals (DAAs) is recommended in order to be eligible for the study.
    e. Liver biopsy must be performed in patients with Hepatitis-B or C infections if the severity assessment of liver disease based on Child-Turcotte-Pugh (CTP) classification indicates all of the following criteria: Serum bilirubin greater than 2, serum albumin less than 3.5, and INR greater than 1.7
    f. Patients with no pathologic evidence of irreversible chronic liver disease such as bridging necrosis and/or significant fibrosis can be eligible for the study.

Pharmacokinetic Drug-Drug Interactions with Antiretroviral (ART) Medications

Preferred ART for Patients Undergoing Autologous Hematopoietic Cell Transplant

- Tier 1: Integrase strand transfer inhibitor (INSTI) based therapy requires no interruption of therapy (example: bictegravir in the combination Biktarvy, dolutegravir (alone or combination product), or raltegravir (alone)). Elvitegravir is contraindicated due to the combination with cobicistat. If combinations of non-boosted elvitegravir are approved, then this INSTI could be considered.
- Tier 2: NNRTI based therapy
- Tier 3: Boosted-PI based therapy

Summary of ART and Drug-Drug Interactions

1. INSTI: bictegravir, dolutegravir, raltegravir, elvitegravir
    a. Dosing
        i. Avoid giving concomitantly with magnesium containing antacids
    b. Toxicity
        i. Raltegravir can exacerbate depression in patients with a previous history of depression.
        ii. Common side effects of diarrhea, headache and nausea has been seen. With raltegravir also there have been issues with hypersensitivity issues with rash and Stevens-Johnson syndrome.
    c. Metabolism
        i. Excreted into feces and urine
    d. Drug Interactions
        i. No interactions with CYP450 co-enzymes
2. Nucleoside Reverse Transcriptase Inhibitors (NRTI): abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir alafenamide fumarate, tenofovir disproxil fumarate, zidovudine
    a. Dosing
        i. Zidovudine (AZT) should not be used in pts receiving hematopoietic cell transplantation
    b. Toxicity
        ii. As a class, adverse effects include lactic acidosis, peripheral neuropathy and hepatic steatosis
    c. Metabolism
        iii. Not extensively metabolized, eliminated renally (except abacavir)
    d. Drug Interactions
        iv. No interactions with CYP450 co-enzymes

3. Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI): delavirdine, doravirine, efavirenz, etravirine, nevirapine, rilpivirine

    a. Dosing

        i. Efavirenz is the preferred agent in this class but it not recommended for use in this trial due to prolonged half life

    b. Toxicity

        ii. All NNRTIs can cause rash, hepatotoxicity and gastrointestinal intolerance

    c. Metabolism

        iii. NNRTs are metabolized hepatically via cytochrome P450 co-enzymes

    d. Drug Interactions

        iv. Nearly all NNRTIs induce CYP3A4 (except for rilpivirine)

4. Protease Inhibitors (PI): atazanavir, darunavir, fosamprenavir, indinavir, nelfinavir, ritonavir, saquinavir, tipranavir

    a. Dosing

        v. All protease inhibitors (except nelfinavir) are typically given in combination with ritonavir to reach optimal pharmacokinetic targets

    b. Toxicity

        vi. All PIs can cause gastrointestinal intolerance, hyperglycemia, lipohypertrophy, osteonecrosis, hepatotoxicity and hyperlipidemia

    c. Metabolism

        i. PIs are metabolized hepatically via cytochrome P450 co-enzymes especially CYP3A4

    d. Drug Interactions

        ii. When given in combination with ritonavir, all PIs are potent CYP3A inhibitors

5. Other

    a. Fusion inhibitor: enfuvirtide
    b. CCR5 inhibitor: maraviroc
    c. Pharmacokinetic enhancer (CYP450 inhibitor): cobicistat (current co-formulations include: Atazanavir-Cobicistat, Darunavir-Cobicistat, Elvitegravir-Cobicistat-Tenofovir DF-Emtricitabine, Elvitegravir-Cobicistat-Tenofovir alafenamide-Emtricitabine, Darunavir-Cobicistat-Tenofovir alafenamide-Emtricitabine)
    d. Attachment inhibitor: ibalizumab-uiyk

APPENDIX E: MANAGEMENT OF ADVERSE REACTIONS

Appendix E

Management of Adverse Reactions to HST-NEETs T Cell Infusions

HST-NEETs infusions are typically well tolerated but adverse reactions may occur early (within 24 hours of infusion) or late (greater than 24 hours after infusion). Early reactions are usually related to the cryopreserved components.

A. Monitoring Post HST-NEETs Infusion

Medical staff will assess for any adverse effects for 1-hour post-infusion. Vital signs should be monitored immediately at end of infusion then at 30 and 60 minutes. Patients should remain on continuous pulse oximetry for at least 30 minutes after infusion.

1. Common, mild complications from HST-NEETs infusion include:

- Mild increase in blood pressure not requiring intervention
- Mild headache
- Mild flushing
- Slight slowing of the heart rate
- Bad taste in mouth

2. Severe early reactions from HST-NEETs infusion are rare but may include:

- Acute pulmonary edema and dyspnea
- Persistent nausea and vomiting
- Sustained or significant increase in blood pressure
- Fever
- Anaphylactic shock
- Bradycardia or cardiac arrest

B. Management of Early Reactions

Most common, mild early reactions do not require intervention.

Management of anaphylaxis: Emergency medications should be ordered in advance and available at the patient's bedside at the time of infusion. Hydrocortisone (or other steroids) should not be administered without approval of physician administering HST-NEETs.

- Hydrocortisone 5-10 mg/kg IV (maximum 500 mg) or methylprednisolone 1-2 mg/kg IV (maximum 125 mg)
- Epinephrine 0.01 mg/kg IM (preferred) or SubQ (maximum 0.5 mg; 0.01 mL/kg of 1 mg/mL solution, maximum of 0.5 mL)

1. In the event of a severe reaction, the patient should be immediately transferred to the intensive care unit for intensive monitoring and intervention, which usually includes blood pressure support, steroids, and management of anaphylaxis.

C. Monitoring and Management of Late Reactions

Late reactions to HST-NEETs infusion may occur but also may be similar to symptoms that occur during neutrophil engraftment.

1. Late reactions to T-cell infusion may be related to a T-cell engraftment syndrome or cytokine release syndrome (CRS), and rarely to contamination of the product.
2. Symptoms and signs of late complications from infusion of T-cell products may include:

Systemic	fever malaise, fatigue myalgias and arthralgias skin rash mimicking acute GvHD general feeling of unwellness disseminated intravascular coagulation (DIC) +/- bleeding macrophage activation syndrome/hemophagocytotic lymphohistiocytosis (HLH) anorexia, nausea, vomiting, diarrhea
Cardiorespiratory	tachycardia blood pressure changes (either hyper- or hypotension) acute pulmonary edema, dyspnea, hypoxia pulmonary infiltrates capillary leak syndrome cardiac dysfunction stress cardiomyopathy (Takotsubo cardiomyopathy) adult respiratory distress syndrome (ARDS)
Hepatic and Renal	weight gain renal impairment azotemia hyperuricemia hepatic impairment – transaminitis, hyperbilirubinemia
Neurological	headache mental status changes confusion delirium hallucinations altered gait seizures encephalopathy mild encephalopathy with reversible splenial lesion syndrome (MERS)

3. Management of Late Reactions

(1) The development of a severe late reaction necessitates immediate notification of the PI and Study Chair AND urgent medical assessment (if an outpatient will necessitate transfer to the hospital). It is important to evaluate the patient and initiate therapy as quickly as possible since rapid deterioration is possible.

(2) Biomarkers: Circulating cytokine levels can serve as biomarkers to diagnose and potentially quantify syndrome severity.

(a) IL-6 signaling is major component of severe cytokine release syndrome

(b) If possible, cytokine levels should be evaluated at inhouse or external (e.g. Viracor) laboratories prior to starting any anticytokine directed therapy. CRP serves as a reliable surrogate for IL-6 bioactivity and levels should be sent pre and post therapy to monitor response

(c) Ferritin may also be used in conjunction with CRP monitoring

(3) The immediate assessment and treatment may also include:

(a) Intensive monitoring (BP, cardiovascular monitoring, pulse oximetry or ABG)

(b) CXR or CT of chest

(c) Steroids – single dose. See #(4) below

(d) Microbiological studies (especially blood cultures and virus studies) and initiation of broad-spectrum antibiotics +/- antivirals should be considered.

(e) Blood pressure/Cardiovascular support – early institution of inotropic medications. Limit fluid resuscitation to the minimum volume needed to support blood pressure, no more than 20 mL/kg, (up to 1,000 mL), if possible.

(f) Fluid management/Renal support – Aggressive diuresis. Institute early involvement of the renal service for fluid management especially if evidence of capillary leak syndrome. Consider infusion of 25% albumin if serum albumin is less than 3 g/dL.

(g) Respiratory support – supplemental oxygen therapy should be initiated for all hypoxemic patients. Patients also may require intubation and mechanical ventilation.

(h) Management of tumor lysis syndrome if there is laboratory evidence it is occurring.

(4) Treatment of the cytokine release syndrome or other T-cell associated adverse reactions must be discussed with the PI and Study Chair. If the situation is emergent, the first dose of steroids can be given prior to consultation with the PI.

In general, the cytokine release syndrome is managed similarly to engraftment syndrome EXCEPT:

- As the patient received an investigational T-cell agent, steroids may not be considered first line therapy and anti-IL-6 directed therapy MAY be given without first starting steroids.

- When managing CRS, anti-IL-6 directed therapy should be initiated before anti-TNFa directed therapy.
- Caution is required when using tocilizumab in the setting of hepatic impairment.

(5) If treatment with a biological agent is necessary, the order of priority will usually be:

- Tocilizumab
- Infliximab(anti-TNFa)
- Etanercept (soluble TNFa receptor inhibitor)

Drug	**Dosing**	**Duration**
Tocilizumab	Less than 30 kg: 8 mg/kg IV Greater than or equal to 30 kg: 4 mg/kg (maximum 800 mg) IV	One dose, with repeat dosing if no improvement observed within 24-48 hours.
Infliximab	10 mg/kg IV	One dose, may give second 3-4 days later
Etanercept	0.4 mg/kg (maximum 25 mg) SubQ	One dose, may give second 3-4 days later

APPENDIX F: REFERENCES

APPENDIX F

REFERENCES

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