BLOOD AND MARROW TRANSPLANT CLINICAL TRIALS NETWORK

An Open-Label, Single-Arm, Multicenter Study of Combination anti-CD3/CD7 Immunotoxin (T-Guard) for Steroid-Refractory Acute Graft-versus-Host Disease

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Version 2.0

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PROTOCOL SYNOPSIS – BMT CTN 1802 PROTOCOL

An Open-Label, Single-Arm, Multicenter Study of T-Guard for Steroid-Refractory Acute Graft-versus-Host Disease

Co-Chairs: John Levine, M.D.
Gabrielle Meyers, M.D.

Study Design: The study is an open-label, single arm Phase III, multicenter trial, which has been designed to evaluate the efficacy and safety of T-Guard treatment in patients with Steroid-Refractory acute Graft versus Host Disease (SR-aGVHD).

The primary analysis will include all patients that initiate T-Guard treatment.

Primary Objective: To assess the rate of Day 28 complete response (CR) in SR-aGVHD patients treated with T-Guard therapy.

Secondary Objectives: Secondary objectives are the following:

1. Evaluate the duration of complete response (DoCR)
2. Estimate the overall survival (OS) at Days 90 and 180
3. Estimate the overall response rate (CR or partial response (PR)) at Days 14, 28, and 56
4. Describe proportions of CR, PR, mixed response (MR), no response (NR), and progression of aGVHD at Days 7, 14, 28, and 56
5. Estimate the cumulative incidence of non-relapse mortality (NRM) at Days 100 and 180
6. Estimate relapse-free survival at Day 180
7. Estimate GVHD-free survival at Days 90 and 180
8. Estimate the cumulative incidence of chronic GVHD (cGVHD) at Day 180
9. Estimate the cumulative incidence of disease relapse/progression at Day 180
10. Describe the incidence of systemic infections
11. Describe the incidence of toxicities
12. Assess the pharmacokinetics of T-Guard
13. Assess the immunogenicity of T-Guard

Exploratory Objectives:

1. Describe corticosteroid-dose (measured in prednisone-equivalent) at baseline, Days 28 and 56 post initiation of T- Guard therapy.
2. Estimate the rate of near-CR (i.e. CR in GI and Liver with only Stage 1 Skin) at Days 28 and 56 post initiation of T-Guard therapy.
3. Describe discontinuation of systemic steroids by Day 180 post initiation of T-Guard therapy.

4. Estimate the incidence of CMV reactivation requiring therapy by Day 180 post initiation of T-Guard Therapy.
5. Estimate the incidence of Epstein-Barr Virus (EBV)-associate lymphoproliferative disorder or EBV reactivation requiring therapy with rituximab by Day 180 post initiation of T-Guard therapy.
6. Describe the incidence of Investigational Medicinal Product (IMP) related SAEs.
7. Evaluate T-cell subsets and Natural Killer (NK) cells at baseline and at Days 0, 2, 4, 6 (just prior to and 4 hours after each T-Guard infusion) and then subsequently at Days 14, 28, 56, 180.
8. Evaluate aGVHD biomarkers at baseline and at Days 7, 14, and 28 post initiation of T-Guard therapy.
9. Describe changes in patient-reported outcomes (PROs) from baseline to Days 28, 56, and 180 post initiation of T-Guard therapy.

Correlatives:

The pharmacokinetics and immunogenicity of T-Guard will be evaluated as referenced in the secondary and exploratory objectives.

Eligibility Criteria:

Adolescents and adults at least 12 years of age at the time of consent who have undergone first allogeneic hematopoietic stem cell transplantation (allo-HSCT) from any donor source using bone marrow, peripheral blood stem cells, or cord blood will be included in this study. Recipients of nonmyeloablative, reduced intensity, and myeloablative conditioning regimens are eligible. Patients must be diagnosed with SR-aGVHD. Steroid refractory (SR) is defined as aGVHD that progressed after 3 days of primary treatment with prednisone (or equivalent) of greater than or equal to 2 mg/kg/day; no improvement after 7 days of primary treatment with prednisone (or equivalent) of greater than or equal to 2 mg/kg/day; or previously was treated with prednisone (or equivalent) of greater than or equal to 1 mg/kg/day and aGVHD has developed in a previously uninvolved organ system. Patients with visceral (GI and/or liver) plus skin aGVHD at prednisone (or equivalent) initiation with improvement in skin GVHD without any improvement in visceral GVHD after 7 days of primary treatment with prednisone (or equivalent) of greater than or equal to 2 mg/kg/day are eligible. Patients must also have evidence of myeloid engraftment (e.g., absolute neutrophil count greater than or equal to 0.5×10^9/L for 3 consecutive days if ablative therapy was previously used). Use of growth factor supplementation is allowed. Patients or an impartial witness (in case the patient is capable to provide verbal consent but not capable to sign the informed consent) should have given written informed consent.

	Exclusion Criteria: Patients will be excluded from study entry if any of the following exclusion criteria exist: Diagnosis of overlap syndrome, that is, with any concurrent features of cGVHD; patients requiring mechanical ventilation, requiring vasopressor support, or requiring hemodialysis; patients who have received any systemic treatment, besides steroids, as upfront treatment of aGVHD OR as treatment for SR-aGVHD; patients with severe hypoalbuminemia, with an albumin of less than or equal to 1 g/dl, a creatine kinase (CK) level greater than 5 times the upper limit of normal; patients with an uncontrolled infection (infections are considered controlled if appropriate therapy has been instituted and, at the time of enrollment, no signs of progression are present). Patients with evidence of relapsed, progressing, or persistent malignancy, or with evidence of minimal residual disease requiring withdrawal of systemic immune suppression are not eligible for this trial. Patients with known hypersensitivity to any of the components murine monoclonoal antibodies (mAb), or Recombinant Ricin Toxin A-chain (RTA), or have received more than one allo-HSCT, or who have known human immunodeficiency virus infection are also excluded from this trial. Pregnant or breastfeeding females, and females of childbearing potential unwilling to use effective birth control from start of treatment until 30 days after the last infusion of T-Guard are not eligible to participate. Male patients who are sexually active and unwilling to use effective birth control from start of treatment until 65 days after the last infusion of T-Guard are not eligible.
Interim Analysis:	This trial will include one interim analysis for futility after 21 patients become evaluable for the primary endpoint. There will be no interim analyses for efficacy.
Treatment Description:	Patients will receive 4 doses of T-Guard treatment, administered intravenously as four 4-hour infusions at least two calendar days apart. Each dose consists of 4 mg/m^2 Body Surface Area (BSA).
Accrual Objective:	The target accrual is 47 patients initiating T-Guard treatment.
Accrual Period:	Approximately 1 year is expected for accrual.
Study Duration:	Patients will be followed for 180 days for a total study duration of approximately 1.5 years.
Safety Monitoring:	The rates of overall mortality and CTCAE Grade 4 or higher capillary leak syndrome (CLS) at Day 30 post treatment initiation will be monitored separately using sequential probability ratio tests (SPRT) for binary data. The SPRTs will contrast a mortality rate of 15% vs. a 30% rate and a Grade 4 or higher CLS rate of 5% vs. a 15% rate.

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CHAPTER 1

1 BACKGROUND AND RATIONALE

1.1 INTRODUCTION

Allogeneic Hematopoietic Cell Transplantation (allo-HSCT) is a potent immunotherapy with curative potential for several hematological disorders (Magenau, Runaas et al. 2016). Improvements in survival following allo-HSCT have led to its increasing use, but the leading cause of non-relapse mortality (NRM) remains graft-versus-host-disease (GVHD) (Alousi, Weisdorf et al. 2009, Major-Monfried, Renteria et al. 2018). Serious infections and impairment of generalized immune function are responsible for GVHD mortality. GVHD incidence and severity depends primarily on donor and recipient matching for human leukocyte antigens and the regimen used for post-grafting immune suppression. The National Institutes of Health (NIH) consensus development project working group recognized 2 main categories of GVHD, each with 2 subcategories. The acute GVHD (aGVHD) category is defined in the absence of diagnostic or distinctive features of chronic GVHD (cGVHD) and includes (1) classic aGVHD occurring within 100 days after transplantation and (2) persistent, recurrent, or late aGVHD (features of GVHD occurring beyond 100 days, often during withdrawal of immune suppression). The broad category of cGVHD includes (1) classic cGVHD (without features or characteristics of aGVHD) and (2) an overlap syndrome in which diagnostic or distinctive features of cGVHD and aGVHD appear together. (Filipovich, Weisdorf et al. 2005, Jagasia, Greinix et al. 2015)

Despite recent advances in the understanding of transplantation immune tolerance, aGVHD is a frequent and major complication of allo-HSCT involving activation of donor T-lymphocytes, which ultimately causes host tissue damage (Holtan SG 2014, LaQuisa 2018). Serious infections, organ failure and impairment of generalized immune function are responsible for aGVHD mortality. The condition involves three target organs, the skin (presenting as inflammatory, maculopapular, erythematous rash), the liver (presenting as hyperbilirubinemia due to cholestatic jaundice) and the gastro-intestinal (GI) tract (presenting as upper and/or lower GI tract manifestations: anorexia with weight loss, nausea, vomiting, diarrhea, severe pain, GI bleeding and/or ileus) (Schoemans, Lee et al. 2018). The diagnosis must occur in absence of cGVHD symptoms (Filipovich, Weisdorf et al. 2005, Jagasia, Greinix et al. 2015). Despite immune suppression prophylaxis, up to 50% of hematopoietic cell transplantation (HCT) recipients will experience grade II-IV aGVHD (Zakias PD 2014, Zeiser, Socie et al. 2016).

Complete responses (CR) to upfront treatment at day 28 of therapy have been reported in 25% to 41% of patients, as defined as regression of skin rash or decrease in the volume of diarrhea and the extent of liver function abnormalities (Hings, Severson et al. 1994, MacMillan, Weisdorf et al. 2002, Deeg 2007). When performing a meta-analysis of prospective studies, including the recently completed REACH1 Study, and of patients reported in the MAGIC database, the expected CR rate at day 28 of therapy is more in the range of 25%-30% (Section 5.1.5). The likelihood of GVHD treatment response decreases with increasing severity of the disease. (Martin, Schoch et al. 1990, Weisdorf, Haake et al. 1990). Treatment responses differ between target organs and may differ considerably between patients. Of particular interest are patients with both visceral and skin aGVHD who have CR of GI or liver GVHD but have persistence of skin rash at less than 25% body surface area (BSA). These patients, who would be considered

to have partial response (PR) by current staging, likely have superior outcomes compared to those patients with persistence of visceral aGVHD. We hypothesize this patient group, with visceral and skin GVHD who meet the criteria for CR except for persistent, low volume skin rash (what we will call near-CR), will have identical outcomes compared to those patients with CR. The response to primary therapy is of central importance, as responses correlate with post-HCT survival.

1.2 THERAPIES FOR ACUTE GVHD

1.2.1 First Line Therapy: Corticosteroids

The mainstay of treatment of aGVHD for over three decades has been high-dose corticosteroids, typically dosed at the prednisone equivalent of 1-2 mg/kg per day (Weisdorf, Haake et al. 1990, Bolanos-Meade and Vogelsang 2004, Bacigalupo 2007, Deeg 2007). However, high dose corticosteroid therapy has several shortcomings, including toxicity issues, such as infection, diabetes, hypertension, osteoporosis, myopathy and avascular necrosis, as well as less than optimal efficacy. This has led to interest for alternate first line therapies e.g. the development of the BMT CTN 1501 clinical trial “A Randomized, Phase II, Multicenter, Open Label, Study Evaluating Sirolimus and Prednisone in Patients with Refined Minnesota Standard Risk, Ann Arbor 1/2 Confirmed Acute Graft-Versus-Host Disease”, comparing outcomes of a non-steroid first-line therapy (sirolimus) for aGVHD. This trial completed enrollment in February 2018 and results are pending publication. While this and other trials are evaluating alternate first line therapies, in the interim and near future, steroids are still expected to be the primary first line therapy for aGVHD. While steroids are considered first line therapy for aGVHD, a significant fraction of the aGVHD population (10-50%) fail to have a clinical response, deeming them steroid refractory (SR). (Deeg 2007, MacMillan, Robin et al. 2015).

1.2.2 Second Line Therapies Background

If the manifestations of GVHD in any organ worsen over 3 days of high-dose steroid treatment or if the involved organs do not improve by 7 days of high-dose steroids therapy, it is unlikely that a response will be achieved in a timely fashion, and secondary therapy should be considered (Deeg 2007). Patients meeting the above criteria are classified as having steroid-refractory aGVHD (SR-aGVHD), and in these patients steroid dosing is not increased since it does not improve survival, (Bacigalupo, van Lint et al. 1983) but rather another immunosuppressive agent is added to the treatment regimen.

Though substantial progress has been made in the field the last decade due to a) advances in selection of patient and donor, b) selection of the most appropriate preparative regimen and c) earlier identification of subpopulations by using biomarkers (Paczesny 2013, Major- Monfried, Renteria et al. 2018), there are still a vast number of patients which end up with SR- aGVHD. The long-term prognosis for this population is unfortunately very poor, with a mortality rate of approximately 70-80% (Weisdorf, Haake et al. 1990, Levine, Logan et al. 2010), because response rates with second-line treatment are low (Deeg 2007, Pidala, Kim et al. 2010, Castilla-Llorente, Martin et al. 2014) and infectious complications due to profound immunosuppression are common.

1.2.3 New Emerging Therapies

Given the dismal overall outcomes in patients with SR-aGVHD many innovative therapies are currently under development (Hill, Alousi et al. 2018). We may conclude that more effective aGVHD immunosuppressive agents will improve remission rates and decrease toxicities, especially in the SR-aGVHD patients, which will result in better survival after allo-HSCT. Ideally, agents should be targeted (i.e. not broadly immunosuppressive), have a favorable side effect profile, should be effective upfront to avoid severe and irreversible tissue damage, and should provide complete and durable responses, without impacting graft versus tumor effects and/or infection risk.

The most promising agents currently being studied include inhibitors of the Janus Kinase pathway (JAK), cytokine modulators, and monoclonal antibodies (mAbs) among others.

Ruxolitinib

The dual JAK1 and JAK2 inhibitor ruxolitinib as well as the JAK1 inhibitor itacitinib have shown efficacy and tolerability in the upfront treatment of aGVHD and SR-aGVHD. The pivotal phase II study of ruxolitinib for SR-aGVHD and steroid-dependent aGVHD showed an overall response rate (ORR) at Day 28 of 57% and 31% CR rate (Jagasia, Zeiser et al. 2018), leading to the FDA approval of this agent on May 24, 2019. The main complications seen with this agent include hematologic toxicity and infections, with 31% of patients discontinuing this agent on trial due to adverse reactions. Larger studies of this agent for SR-aGVHD are underway (REACH 2, ClinicalTrials.gov identifier:NCT0291326).

Early studies of the JAK1 inhibitor itacitinib for aGVHD shows tolerability and suggestion of activity. The Phase I trial of itacitinib combined with steroids for treatment naïve and SR- aGVHD was reported at the 2016 American Society of Hematology Meeting (Schroeder et al, Blood 2016, Abstract 390), with ORR of 64.7% in SR-aGVHD. GRAVITAS-301 is a phase III trial currently enrolling for front-line treatment of aGVHD (ClinicalTrials.gov identifier: NCT03139604).

AAT

The serine protease inhibitor alpha-1 antitrypsin (AAT) has been shown to impact T cell repertoire and inflammatory cytokines, and levels of the naturally occurring AAT in donors was found to impact GVHD outcomes (Marcondes, Karoopongse et al. 2014). This data led to the Phase I/II dose escalation study of AAT for treatment of SR-aGVHD, which showed optimistic Day 28 response rates (ORR 65%, CR 35%) and good tolerance, supporting additional trials with this agent (Marcondes, Hockenbery et al. 2016).

mAbs

Finally, our experience and ability to manufacture (mAbs) has rapidly expanded, with multiple immunomodulatory mAbs (Floisand, Lundin et al. 2017, Hill, Alousi et al. 2018) currently under investigation for the treatment of aGVHD.

The goal of any new therapy for aGVHD is to improve remission rates and minimize toxicities, especially in the SR patient, thereby leading to improved survival after allo-HSCT. Each of the agents highlighted above, and others being studied for SR-aGVHD, have certain advantages

and disadvantages, but maintain a suboptimal response rate. Therefore, a very high priority remains for development of an effective and well tolerated therapy for SR-aGVHD.

In the proposed study a new immunotoxin is investigated in patients with SR-aGVHD that may meet all of the criteria for an ideal agent based on immunosuppressive profile reported on 20 patients in a Phase I/II clinical trial (Groth, van Groningen et al. 2017). Anti-CD3/anti-CD7 antibody treatment in patients with severe SR-aGVHD resulted in high day 28 CR rate of 50%, which compares favorably with historical controls (20-30%), and a high Day 180 overall survival (OS). In addition, the short treatment course (4 treatments given every 48 hours) allows for a rapid response and fast restoration of the immune system.

1.3 T-GUARD

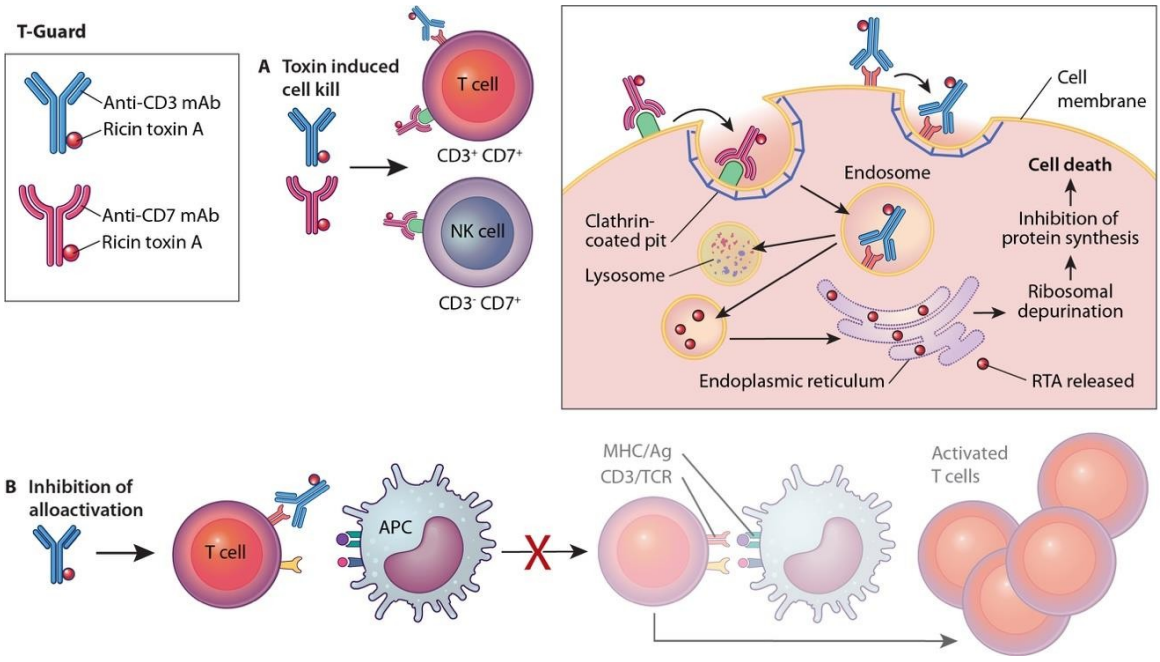
The investigational medicinal product (IMP) in this study, T-Guard, is an immunotoxin-combination, consisting of two equal amounts (w/w) of the murine mAbs SPV-T3a (anti-CD3, IgG2b) and WT1 (anti-CD7, IgG2a), each conjugated to the Ricin Toxic A (RTA) chain: SPV-T3a-RTA and WT1-RTA. This particular immunotoxin-combination harbors multiple mechanisms of action (Figure 1-1).

Both the anti-CD3 and anti-CD7 mAbs act as chaperones that bring the toxic RTA payload into the target cells after binding to the surface antigens on T cells (CD3+ and CD7+) and Natural Killer (NK) cells (CD7+ and CD3-). Once inside the cell, the bond between these mAbs and RTA toxin is broken, thereby releasing free RTA into the cytoplasm. The RTA toxin then irreversibly inhibits protein synthesis by means of a catalytic reaction culminating in programmed cell death (apoptosis) of T cells, with a preference for the recently-activated ones, and NK cells (see also 1.3.1 Pre-clinical data). RTA toxin does not enter the cell autonomously. Because the RTA toxin is unable to bind or enter the cell autonomously, immunotoxins are only hazardous to cells capable of binding and internalizing the mAbs (Wayne, Fitzgerald et al. 2014), therefore mitigating toxicity to bystander cells (van Oosterhout, van Emst et al. 2000).

Additionally, two other anti-T-cell immunotoxins have been clinically tested as a single-agent for the in vivo prevention/treatment of aGVHD: anti-CD5 immunotoxin H65-RTA (Xomazyme CD5), which was constructed with RTA chain, and anti-CD25 directed denileukin diftitox (Ontak), a recombinant fusion protein linking cytokine interleukin (IL)-2 to truncated diphtheria toxin. Although these single-agent immunotoxins showed promising initial results, when tested as second-line treatment for SR-aGVHD, they appeared not to be superior than available aGVHD treatments. (Byers, Henslee et al. 1990, Krance, Heslop et al. 1993, Hings, Severson et al. 1994, Phillips, Nevill et al. 1995, Martin, Nelson et al. 1996, Ho, Zahrieh et al. 2004, Shaughnessy, Bachier et al. 2005).

One of the reasons T-Guard might be more successful is that the simultaneous targeting of two or more antigens on the same target cell may result in a synergistic toxicity (see also 1.3.1 Pre-clinical data). Moreover, CD3 antibody binding to the CD3/T cell receptor (CD3/TCR) complex results in competitive inhibition and internalization of the antibody-receptor complex, thus blocking T cell activation directly and may trigger activation-induced cell death of activated T cells.

Figure 1-1: T-Guard Mechanism of Action



1.3.1 Pre-Clinical Data

We hypothesize that T-Guard might have a role in treatment and/or prevention of clinical GVHD based on the pre-clinical immunological profile.

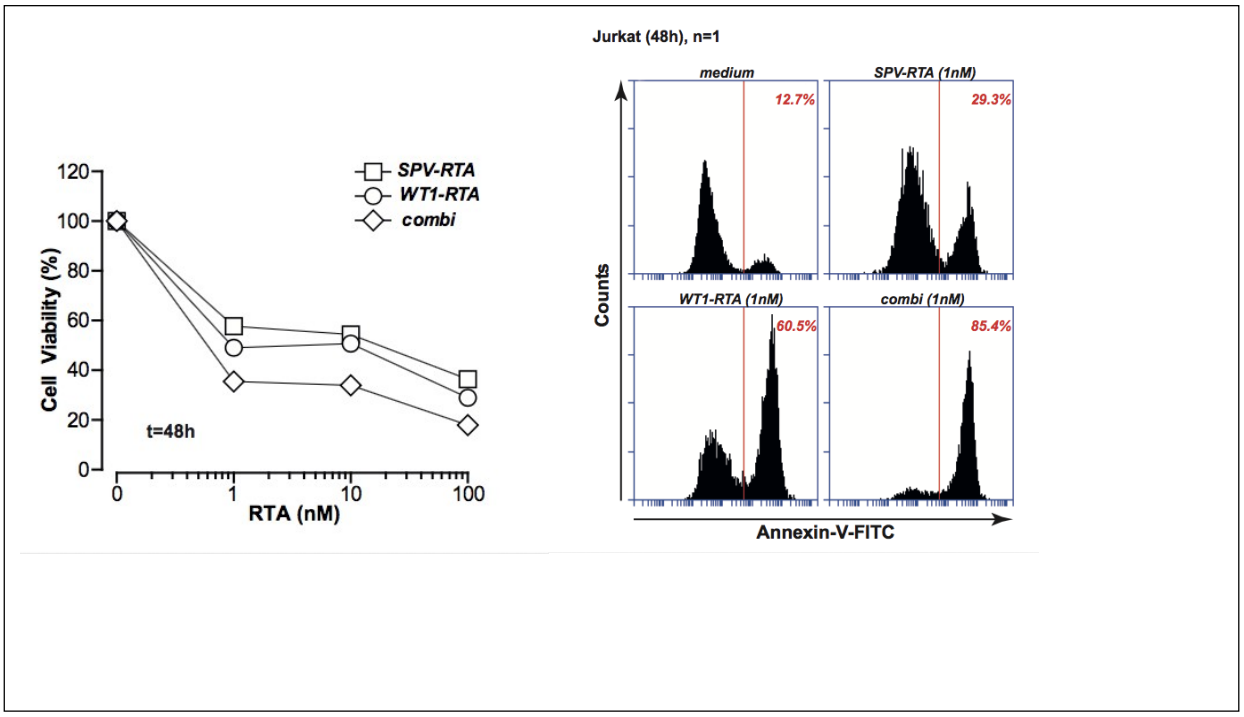
1.3.1.1 Synergistic Apoptosis of a Combination of Anti-T-Cell Immunotoxins

Several anti-T cell mAbs were conjugated to RTA and evaluated for their efficacy, alone and in combination, to eliminate or neutralize activated T cells in vitro. These experiments led to the selection of an immunotoxin-combination consisting of SVP-T3a-RTA (anti-CD3) and WT1-RTA (anti-CD7). This particular combination, having the working name ‘T-Guard’, was more effective at inducing T cell apoptosis than each of the individually tested immunotoxins. Figure 1-2 shows % cells viability of Jurkat cells treated with either SPV-T3a-RTA and WT1- RTA or in combination (half a dose each).

1.3.1.2 Cytokine Modulation by T-Guard

Both SPV-T3a and WT1 deliver the toxic RTA-payload inside the T cells, and the SPV-T3a modulates the CD3/TCR complex (see above). Notably, SPV-T3a is particularly well suited for *in vivo* use, as this anti-CD3 mAb does not stimulate T cells (Land, Hillebrand et al. 1988, Smely, Weschka et al. 1990, Frenken, Koene et al. 1991, Woodle, Thistlethwaite et al. 1991, Anasetti, Martin et al. 1992, Knight, Kurrle et al. 1994). This strongly reduces the occurrence of cytokine release syndrome (CRS), a potentially life-threatening complication frequently associated with the clinical use of non-conjugated immunosuppressive anti-CD3 mAbs or Anti- Thymocyte Globulin (ATG).

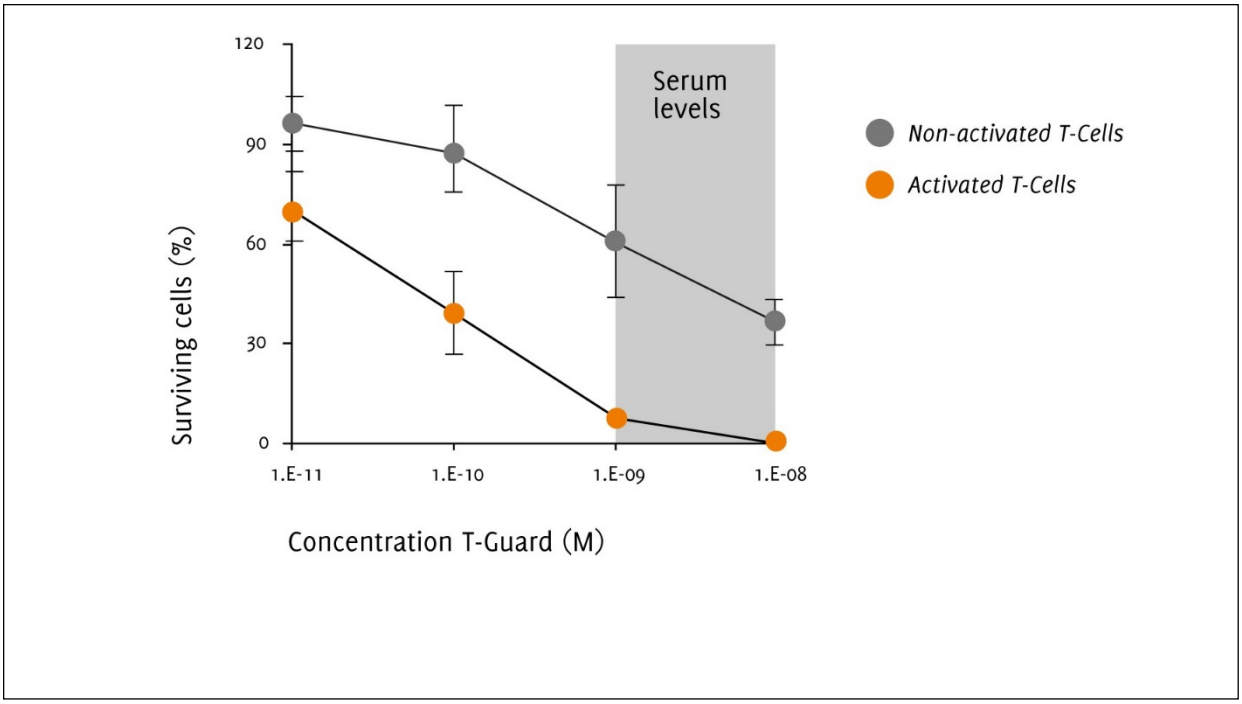
Figure 1-2: Synergistic cell kill by the T-Guard combination



1.3.1.3 Preferential Targeting of Activated T Cells over Non-Activated and Anti-Viral T cells

The SPV-T3a-RTA and WT1-RTA immunotoxin-combination preferentially kills recently activated T cells (van Oosterhout, van Emst et al. 2000). Figure 1-3 depicts the outcome of an *ex vivo* experiment, in which non-activated and phytohemagglutinin (PHA)-activated Peripheral Blood Mononuclear Cells s were incubated with various concentrations of T-Guard. Incubation with clinically relevant T-Guard concentrations (10^{-9} M – 10^{-8} M) results in a reduction of the number of activated T cells to less than 1%, while 35% of their non-activated counterparts survived. Flow cytometric analysis revealed that the higher vulnerability of recently activated T cells could most likely be attributed to a strong increase in CD7 membrane expression. This upregulation of CD7 expression upon recent T cell activation with either PHA, anti-CD3 mAbs or in a Mixed Lymphocyte Reaction has been described by others as well (Heinrich, Gram et al. 1989, Akbar, Amlot et al. 1990, Amlot, Tahami et al. 1996).

Figure 1-3: Preferential targeting of activated T cells (ex vivo experiment)



It is also noteworthy that, apart from recent T cell activation, CD7 expression also seems to be regulated by differentiation stage, with lower CD7 expression on mature effector memory T cells (Reinhold, Abken et al. 1993, Amlot, Tahami et al. 1996, Aandahl, Sandberg et al. 2003, Appay, van Lier et al. 2008). This may portend clinical benefit given that virus-specific T cells reside primarily in the effector memory compartments (Gamadia, Rentenaar et al. 2001, Aandahl, Quigley et al. 2004, Appay, van Lier et al. 2008, Shindo, Kim et al. 2013), and may therefore be relatively spared by T-Guard treatment.

1.3.1.4 NK Cells Apoptosis

Aside from T cells, the immunotoxin-antibody combination WT1-RTA also targets NK cells (CD3-/CD7+). In the Phase I/II clinical study using T-Guard, NK cells demonstrated the same depletion pattern as T cells, an early and quick depletion during the seven-day treatment period, followed by a rapid repopulation of the NK cell compartment (Groth, van Groningen et al. 2018). The NK cell depletion is thought to contribute to controlling established aGVHD disease, as NK cells may aggravate the severity of aGVHD through cytokine production, e.g. Interferon (IFN)-gamma (Gill, Olson et al. 2009).

1.3.2 Clinical data

1.3.2.1 Clinical Experience with T-Guard in Patients with SR-aGVHD

T-Guard has been evaluated in two clinical trials: as third-line therapy in an investigator-initiated dose escalation-study in seven patients suffering from SR-aGVHD (van Oosterhout, van Emst et al. 2000, van Oosterhout, van Emst et al. 2001) and as second-line therapy in a Phase I/II study in 20 patients with SR-aGVHD (Groth, van Groningen et al. 2017). Results from these studies are summarized below.

1.3.2.2 T-Guard Dose Escalation Study as Third Line for SR-aGVHD Patients

The main purpose of the investigator-initiated dose escalation study was to test the safety and pharmacokinetics (PK) of T-Guard. A total of seven male Caucasian patients, with a median age of 47 with SR-aGVHD that had failed second line therapy were included. Three of these patients (43%) had aGVHD Grade IV, 3 patients (43%) Grade III and 1 patient Grade II (14%). Six patients (86%) had visceral organ involvement, including four patients (57%) with liver involvement and two patients (29%) with GI involvement. One patient (14%) had grade IV aGVHD of the skin. Before T-Guard treatment, all patients received an initial course of steroids at 1-2mg/kg/day for at least 7 days, followed by 1 gram of methylprednisolone equivalent per day or at least 3 days to treat SR-aGVHD. The first two patients started with T-Guard 2mg/m^2 infused every 48 hours x 2 doses, followed by 4mg/m^2 every 48 hours x 2 doses. There were no toxicities associated with the T-Guard treatment, and therefore the next five patients were scheduled to receive 4mg/m^2 every 48 hours x 4 doses.

The infusions were well tolerated, and biologic and clinical responses, as further described below, were achieved. Given the activity and tolerance of the 4mg/m^2 every 48 hours x 4 doses schema, no further dose escalation was completed. The study team was concerned for capillary leak syndrome (CLS), which is a known complication of ricin-based immunotoxins. However, none of the patients had any severe toxic side effects (grade 3 or higher Common Terminology Criteria for Adverse Events (CTCAE) 4.3) associated with RTA-based immunotoxins, like severe CLS and rhabdomyolysis. Regarding Treatment Emerging Adverse Events (TEAE), the most frequent reported adverse events (AE) were: pyrexia (43%) and headache (29%) followed by pain, edema, aphasia, dizziness, epilepsia, diarrhea, hemorrhoids, paralytic ileus and protein losing gastroenteropathy (all 14%).

PK analysis showed that the 4mg/m^2 every 48 hours x 4 doses schedule resulted in T-Guard in vivo serum peak concentration (C_{max}) of around 1.5 µg/ml (~10^{-8}M). On the basis of in vitro experiments, the C_{max} attained results in a 60-80% occupation of the target antigens CD3 and CD7 and corresponds with the optimal in vitro concentration for a specific elimination of antigen-positive target cells (Figure 1-3).

Four patients were evaluated for biologic response with flow cytometric quantitation of NK and T cell levels. All of these patients showed a rapid and profound reduction in circulating T cells and NK cells, with additional doses leading to further reduction (less than 10-20%) of initial levels of circulating NK and T cells. With this prompt and marked decline in NK and T cells there was clinical improvement in GVHD, with four of the seven patients having clinically meaningful and rapid reduction in their GVHD. Five out of 7 patients (71%) responded. One patient (14%) was in complete remission, 4 patients (57%) had a PR. To date 6 patients died, two during the first eight days of treatment with T-Guard. All patient deaths were related to multisystem organ failure and opportunistic infections.

Since the dose level of T-Guard at 4 mg/m^2 x 4 doses generated biologically relevant serum concentrations (C_{max} of 1.36 ± 0.27 µg/mL) which resulted in clear biological and clinical responses without inducing severe acute toxicities, the dosage was not further increased to prevent potentially dangerous dose limiting toxicities (DLTs).

1.3.2.3 Single-arm, Phase 1/2 Clinical trial in second line SR-aGVHD Patients

Following the promising results of T-Guard for the indication SR-aGVHD in the investigator-initiated dose escalation study, a Phase 1/2 study XEN/TG-001 was conducted (Groth, van Groningen et al. 2018). The study was designed to evaluate the safety and efficacy of T- Guard in treating SR-aGVHD patients who were refractory to first-line therapy. Regarding the IMP (T-Guard), a switch was made to non-glycosylated bacterial produced recombinant RTA. In total 20 patients were treated with T-Guard with a dose of 4 mg/m^2 every other day x 4 doses. All patients had SR grade II to IV aGVHD. Seventeen of these patients (85%) had severe SR-aGVHD, and all 20 patients had visceral involvement; 18 GI (90%) and 5 liver (25%) involvement. Sixteen patients (80%) had 2 or more organs involved. A validated two biomarker algorithm classified the majority of patients (11/20) as high-risk.

The Day 28 ORR (primary endpoint), defined as having a CR or PR without the need for initiation of other treatments due to insufficient response, was 60%. Day 28 CR was 50% and PR 10%. The Day 180 OS was 60%. The outcomes achieved were very favorable compared with the historical standard of care (SoC) data at the participating centers (Day 28 CR less than 20% and Day 180 OS 29%).

1.3.2.4 Safety

The most common side-effects described for RTA-based immunotoxins are vascular leakage and myalgia, the latter being often associated with an increase in serum creatine kinase (CK). Moreover, the systemic administration of anti-CD3 antibodies may result in the activation of T-cells leading to CRS. In both trials T-Guard was overall well-tolerated. Mild infusion related reactions, such as mild chills, were documented. However, no acute toxicity reactions seen were greater than Grade 1. The most commonly reported AEs considered to be related to T- Guard administration were hypoalbuminemia and thrombocytopenia.

The potentially drug-associated side effects occurring in more than one patient consisted of (further) decrease in platelet count (thrombocytopenia), hypoalbuminemia and thrombotic microangiopathy. Regarding thrombocytopenia and hypoalbuminemia, the patients in the study already had a low platelet count and albumin level due to aGVHD and medication. Nevertheless, in several patients, a further decrease was observed during the treatment period. According to the Principal Investigators, thrombocytopenia and hypoalbuminemia were well-manageable. TEAEs from the overall study are outlined in Table 1-1 from the Phase I/II Trial.

Table 1-1: Most Frequently Reported System Organ Class (≥10 Patients in Total) and Preferred Term (≥5 Patients in Total) for TEAEs, Overall Study

System organ class/ Preferred term	Total (N=20) n (%)
Any adverse event	20 (100.0%)
Infections and infestations	19 (95.0%)
Upper respiratory tract infection	5 (25.0%)
Metabolism and nutrition disorders	17 (85.0%)

System organ class/ Preferred term	Total (N=20) n (%)
Hypoalbuminemia	8 (40.0%)
Hyperglycemia	7 (35.0%)
Hypokalemia	5 (25.0%)
Hypophosphatasemia	5 (25.0%)
General disorders and administration site conditions	16 (80.0%)
Edema peripheral	8 (40.0%)
Pyrexia	8 (40.0%)
Fatigue	6 (30.0%)
Gastrointestinal disorders	15 (75.0%)
Nausea	5 (25.0%)
Investigations	13 (65.0%)
Blood bilirubin increased	6 (30.0%)
White blood cell count decreased	5 (25.0%)
Musculoskeletal and connective tissue disorders	13 (65.0%)
Muscular weakness	5 (25.0%)
Myopathy	5 (25.0%)
Vascular disorders	12 (60.0%)
Capillary leak syndrome	8 (40.0%)
Blood and lymphatic system disorders	11 (55.0%)
Anemia	6 (30.0%)
Thrombocytopenia	6 (30.0%)
Nervous system disorders	11 (55.0%)

In total 29 Serious Adverse Events (SAE) were reported in 14 patients (Table 1-2). Of those SAEs, 23 were reported as severe, 4 as moderate and 2 as mild. Eight (8) patients died during the course of the trial. The causes of death were determined to be refractory aGVHD, infections or the combination of the two. All deaths were reported as not related to T-Guard. No deaths occurred before 6 months (Day 180) due to relapse of the underlying disease.

Table 1-2: Summary of Adverse Events Potentially Related to Treatment (Groth, van Groningen et al. 2018)

Grade 2[a]	Grade 3	Grade 4
Anemia (1)[b]	Thrombocytopenia (3)	Thrombocytopenia (5)
Abdominal pain (1)	Neutropenia (1)	
Thrombocytopenia (1)	Elevated bilirubin (2)	
Neutropenia (1)	Myopathy (1)	
Microangiopathy (1)	Microangiopathy (1)	

Grade 2[a]	Grade 3	Grade 4
Chills (2)	Hypoalbuminemia (1)	
Capillary leak syndrome (1)		
Hypoalbuminemia (1)		

Grading of each AE is based on version 4.0 of the CTCAE, with the exception of CLS, which was graded using the system described by Messmann et al. (Messmann, Vitetta et al. 2000)

The numbers in parentheses refer to the number of patients who experienced the indicated adverse event.

The overall conclusion of the Phase 1/2 study is that T-Guard was well tolerated and can be safely administered in patients with SR-aGVHD. Additionally, the response rates, in particular CR rates, at Day 28 were markedly superior (CR 50%) to historical controls and the results of emerging therapies (CR 20-35%).

1.3.3 Justification for the Proposed Dose Regimen for this Phase III Clinical Trial Using T-Guard as First Line SR-GvHD Treatment

The proposed dose for the T-Guard Phase 3 study is 4 mg/m^2 / dose q 48 hours x 4 doses. This dose is based on the following data:

In the investigator-initiated dose-escalation study (van Oosterhout, van Emst et al. 2000, van Oosterhout, van Emst et al. 2001) it was determined that the second dose level of 4 mg/m^2/dose q 48 hours x 4 doses delivered positive biological and clinical responses, without inducing any severe acute toxicities.

PK analysis showed that the 4 mg/m^2 x 4 doses schedule led to T-Guard *in vivo* serum C_{max} of around 1.5 µg/ml (~10^{-8}M). On basis of *in vitro* experiments, the C_{max} attained results in a 60-80% occupation of the target antigens CD3 and CD7. It has been shown in *in vitro* studies that this concentration is very effective in specifically eliminating antigen-positive target cells by RTA-based immunotoxins (Derocq, Laurent et al. 1987, Preijers, De Witte et al. 1988, Preijers, Tax et al. 1988, Preijers, Tax et al. 1988, Herrera, Farah et al. 2000).

Given the 50% Day 28 CR rate observed in the T-Guard Phase 1/2 study, it could be argued that the clinical benefit may be further optimized by administering a higher T-Guard dose. When judging the response rate, though, consideration is given to the high incidence of serious comorbidities in this severely ill population. Four of the 20 patients of the T-Guard Phase 1/2 study died before Day 28 due to comorbidities; two patients died from sepsis (non- treatment related), one patient died in the treatment week with uncontrolled viral infections, and another patient had vanishing bile duct syndrome with very rare aberrant bile ducts at treatment start suggestive for co-existing liver disease of different etiology. When these four patients are censored, the Day 28 response rate increases to 63% CR (10/16) and 75% ORR (12/16), respectively. In the Phase 1/2 study, the adverse events judged to be potentially treatment-related were clinically manageable and included 7 mild cases (35%) and 1 moderate case (5%) of CLS.

To put the currently applied T-Guard dose in perspective, the Maximum Tolerated Dose as

determined for similar RTA-based immunotoxins typically lies between 4 and 8 mg/m^2 with capillary leakage being the most common dose limiting toxicity (Amlot, Stone et al. 1993, Stone, Sausville et al. 1996, Engert, Diehl et al. 1997, Messmann, Vitetta et al. 2000, Schnell, Staak et al. 2002, Schindler, Gajavelli et al. 2011). Thus, given concern for increased toxicity due to the immunotoxin at higher doses, the Food and Drug Administration was in agreement with the proposed dose selection. In conclusion, the observed positive benefit-risk profile of the proposed dose regimen is considered sufficient to focus on confirming the safety and efficacy of T-Guard's current 4 x 4 mg/m^2 dose in a pivotal Phase 3 clinical study. In the proposed pivotal Phase 3 study, further data will be collected to investigate if biomarkers can be identified that correlate with clinical efficacy and safety.

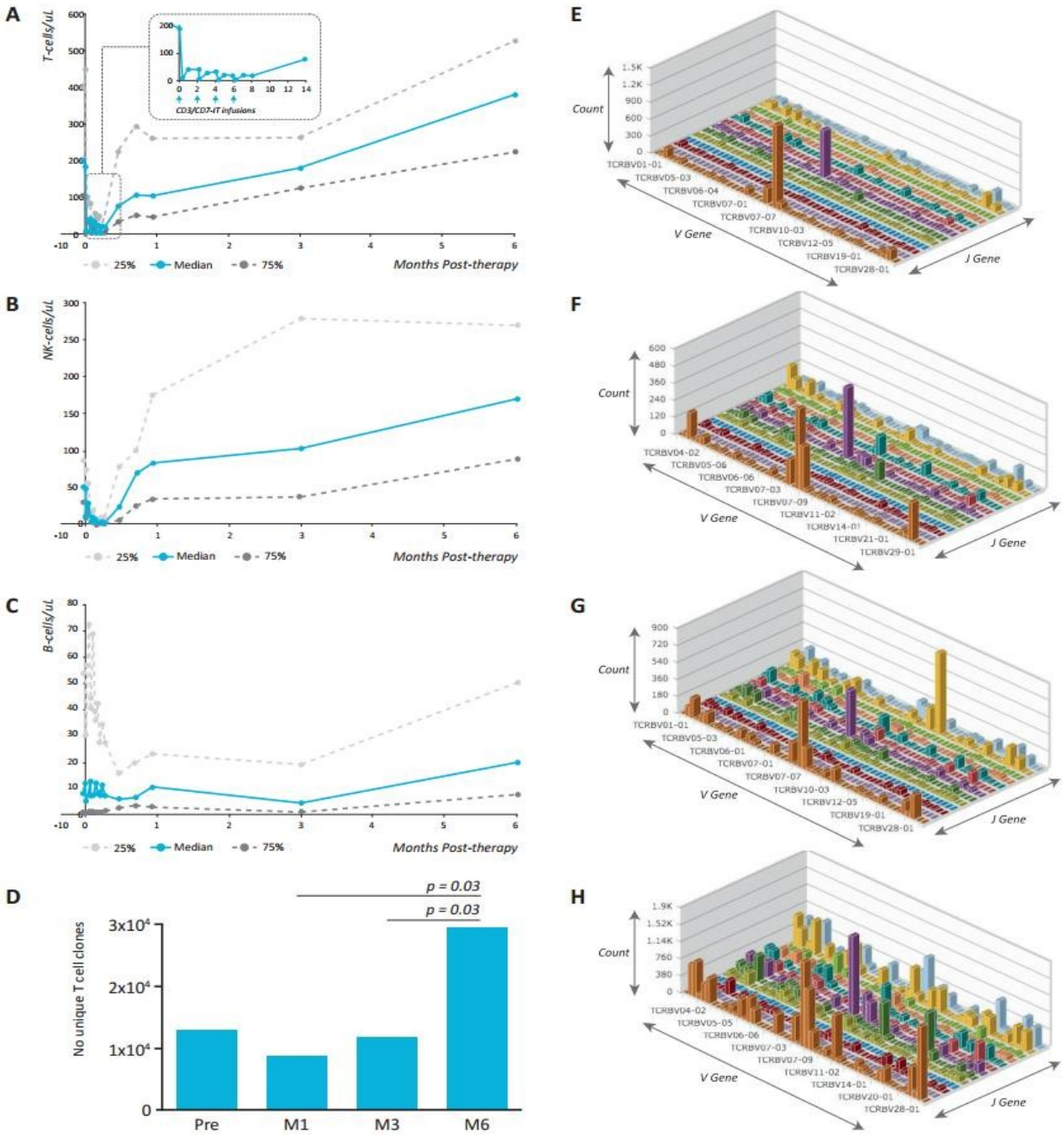
1.4 STUDY RATIONALE

T-Guard has a rapid onset, preferential killing of activated T cells, and short half-life, leading to depletion of allo-reactive T cells and quick post-treatment reconstitution of the immune system. In the Phase 1/ 2 trial with T-Guard, peripheral blood samples were analyzed before and after treatment. As expected, treatment with T-Guard led to profound depletion of T cells and NK cells, with rapid recovery starting as early as 14 days following treatment. (Figure 1- 4). No specific treatment-induced changes in the relative proportions of naïve, memory, effector and effector memory type of T-cells were found before and after treatment, and regulatory T cells showed normal variation throughout the study. The study of T cell diversity found low T-cell diversity before T-Guard treatment, which further declined by month one post- treatment, likely due to reduction in T cell numbers. This was followed, at months two through six, with a steady rebound in the T-cell diversity. By 180 days post-treatment the T-cell repertoire was both diverse and expanded, with several new polyclonal T cell populations found.

The proposed model is that immunodepleting the vast majority of alloreactive T cells will shift the immune balance to a more tolerogenic state in SR-aGVHD patients, resulting in better disease control and an improved survival. We hypothesize depleting NK cells will contribute to the tolerogenic state by suppressing cytokine release and inflammation.

Given the high mortality and limited treatment options for patients with SR-aGVHD, we propose conducting a Phase III single arm open label multicenter trial to evaluate the safety and efficacy of T-Guard. A single arm study is justified given that this is a rare disease without an established SoC, yet with compelling historical data on response and survival rates. The 50% Day 28 CR rate observed in the T-Guard Phase I/II study compares favorably to the 31% CR rate seen with ruxolitinib in the REACH1 trial. This well-controlled Phase III trial will evaluate the hypothesized improvement in Day 28 CR rates in combination with the proposed PK, immunogenicity, and biomarker studies while evaluating durability of CR and OS.

Figure 1-4: T-Guard Observed Immune Reconstitution



T-Guard induces rapid immune reconstitution with a diverse T cell repertoire. (A-C) Time course of the median T cell count (A), median NK cell count (B), and median B cell count (C) for all patients. In each plot, the blue line represents the median value, and the lower and upper gray dotted lines represent the 25th and 75th percentiles, respectively. (D) Summary of the absolute number of unique T cell clones before administration of T-Guard (Pre) and at 30, 90, and 180 days after treatment. The number of unique T cell clones was measured using the total number of unique CDR3 sequences. The P values are based on the Wilcoxon matched-pairs signed-rank test. The significant increase in unique T cell clones at Day 180 after T-Guard treatment reflects an increase in the diversity of expanded T cells. (E-H) Representative histograms

showing the T cell repertoires in a single patient before T-Guard treatment (E) and at 30 days (F), 90 days (G), and 180 days (H) after therapy.

1.5 INCLUSION OF CHILDREN AGE 12-17

This study allows children aged 12-17 to enroll. This study will be a first in the evaluation of the safety and effectiveness of T-Guard in a pediatric population. Only adolescent children, who are most similar to adult patients, will be treated to mitigate risk. As in adults, SR-aGVHD is a highly morbid and life-threatening complication of transplant. Ruxolitinib is now FDA- approved for treatment of SR-aGVHD in ages 12 and above. However, the 50% Day 28 CR rate observed in the T-Guard Phase 1/2 study compares favorably to the 31% CR rate seen with ruxolitinib in the REACH1 trial. Ruxolitinib is also only available as an oral compound and given as an extended treatment course, which may not be tolerable for SR-aGVHD patients with GI involvement. Given the high rates of GI involvement in SR-aGVHD and short IV course of T-Guard, enrollment of children ages 12 and above on this trial is justified.

CHAPTER 2

2 STUDY DESIGN

2.1 STUDY OVERVIEW

This is a Phase III, open label, single arm, multicenter trial designed to evaluate the efficacy of T-Guard treatment in patients with SR-aGVHD.

2.2 HYPOTHESIS AND SPECIFIC OBJECTIVES

2.2.1 Primary Hypothesis

The primary hypothesis is that T-Guard treatment will improve the Day 28 complete remission (CR) rate in patients with steroid-refractory (SR) acute GVHD (aGVHD).

2.3 STUDY OBJECTIVES

2.3.1 Primary Objective

The primary objective of this trial is to assess the rate of Day 28 CR in SR acute GVHD patients treated with T-Guard treatment.

2.3.2 Secondary Objectives

1. Evaluate the duration of complete response (DoCR).
2. Estimate overall survival (OS) at Days 90 and 180 post initiation of T-Guard treatment.
3. Estimate the overall response rate (CR or partial response (PR)) at Days 14, 28, and 56 post initiation of T-Guard treatment.
4. Describe proportions of CR, PR, mixed response (MR), no response (NR), and progression of aGVHD at Days 7, 14, 28, and 56 post initiation of T-Guard treatment.
5. Estimate the cumulative incidence of NRM at Days 100 and 180 post initiation of T- Guard treatment.
6. Estimate relapse-free survival at Day 180 post initiation of T-Guard treatment.
7. Estimate GVHD-free survival at Days 90 and 180 post initiation of T-Guard treatment.
8. Estimate the cumulative incidence of cGVHD at Day 180 post initiation of T-Guard treatment.
9. Estimate the cumulative incidence of disease relapse/progression at Day 180 post initiation of T-Guard treatment.
10. Describe the incidence of systemic infections.
11. Describe the incidence of toxicities.
12. Assess the pharmacokinetics of T-Guard.
13. Assess the immunogenicity of T-Guard.

2.3.3 Exploratory Objectives

1. Describe corticosteroid-dose (measured in prednisone-equivalent) at baseline, Days 28 and 56 post initiation of T-Guard treatment.
2. Estimate the rate of near-CR (i.e. CR in GI and Liver with only Stage 1 Skin) at Days 28 and 56 post initiation of T-Guard treatment.
3. Describe discontinuation of systemic steroids by Day 180 post initiation of T-Guard treatment.
4. Estimate the incidence of CMV reactivation requiring treatment by Day 180 post initiation of T-Guard treatment.
5. Estimate the incidence of EBV-associated lymphoproliferative disorder or EBV reactivation requiring treatment with rituximab by Day 180 post initiation of T-Guard treatment.
6. Describe the incidence of IMP related SAEs.
7. Evaluate T-cell subsets and NK-cells at baseline and at Days 0, 2, 4, 6 (just prior to and 4 hours after each T-Guard infusion) and then subsequently at Days 14, 28, 56, and 180.
8. Evaluate aGVHD Biomarkers at baseline and at Days 7, 14 and 28 post initiation of T- Guard treatment.
9. Describe changes in patient-reported outcomes (PROs) from baseline to Days 28, 56 and 180 post initiation of T-Guard treatment.

2.4 PATIENT ELIGIBILITY

2.4.1 Inclusion Criteria

To be eligible to participate in this study, patients must meet the following eligibility criteria:

1. Patient must be at least 12.0 years of age at the time of consent.
2. Patient has undergone first allo-HSCT from any donor source using bone marrow, peripheral blood stem cells, or cord blood. Recipients of nonmyeloablative, reduced intensity, and myeloablative conditioning regimens are eligible.
3. Patients diagnosed with SR-aGVHD after allo-HSCT. SR is defined as aGVHD that:

    - Progressed after 3 days of primary treatment with prednisone (or equivalent) of greater than or equal to 2 mg/kg/day
    - No improvement after 7 days of primary treatment with prednisone (or equivalent) of greater than or equal to 2mg/kg/day
    - Patients with visceral (GI and/or liver) plus skin aGVHD at prednisone (or equivalent) initiation with improvement in skin GVHD without any improvement in visceral GVHD after 7 days of primary treatment with prednisone (or equivalent) of greater than or equal to 2mg/kg/day
    - Previously was treated with prednisone (or equivalent) of greater than or equal to 1mg/kg/day and aGVHD has developed in a previously uninvolved organ system

Progression and no improvement are defined in Section 3.1. Improvement or progression in organs is determined by comparing current organ staging to staging at initiation of prednisone (or equivalent) treatment. Staging is performed per MAGIC criteria (Appendix C).

4. Evidence of myeloid engraftment (e.g., absolute neutrophil count greater than or equal to 0.5 × 10^9/L for 3 consecutive days if ablative therapy was previously used). Use of growth factor supplementation is allowed.

5. Patients or an impartial witness (in case the patient is capable of providing verbal consent but not capable of signing the informed consent form (ICF)) should have given written informed consent. For patients less than 18 years of age, a written informed consent of the parents or guardian and written assent of the patient will be obtained, per the local requirements.

2.4.2 Exclusion Criteria

Patients will be excluded from study entry if they meet any of the following exclusion criteria:

1. Patients who have been diagnosed with overlap syndrome, that is, with any concurrent features of cGVHD.

2. Patients requiring any of the following: mechanical ventilation, vasopressor support, or hemodialysis.

3. Patients who have received any systemic treatment, besides steroids, as upfront treatment of aGVHD OR as treatment for SR-aGVHD.

4. Patients who have severe hypoalbuminemia, with an albumin of less than or equal to 1 g/dl.

5. Patients who have a CK level of greater than 5 times the upper limit of normal.

6. Patients with uncontrolled infections. Infections are considered controlled if appropriate therapy has been instituted and, at the time of enrollment, no signs of progression are present. Progression of infection is defined as hemodynamic instability attributable to sepsis, new symptoms, worsening physical signs or radiographic findings attributable to infection. Persisting fever without other signs or symptoms will not be interpreted as progressing infection.

7. Patients with evidence of relapsed, progressing or persistent malignancy.

8. Patients with evidence of minimal residual disease requiring withdrawal of systemic immune suppression.

9. Patients with known hypersensitivity to any of the components murine monoclonal antibodies (mAb) or Recombinant Ricin Toxin A-chain (RTA).

10. Patients who have received more than one allo-HSCT.

11. Patients with known human immunodeficiency virus infection.

12. Female patients who are pregnant, breast feeding, or, if sexually active and of childbearing potential, unwilling to use effective birth control from start of treatment until 30 days after the last infusion of T-Guard.

13. Male patients who are, if sexually active and with a female partner of childbearing potential, unwilling to use effective birth control from start of treatment until 65 days after the last infusion of T-Guard.

14. Patients with any condition that would, in the investigator's judgment, interfere with full

participation in the study, including administration of study drug and attending required study visits; pose a significant risk to the patient; or interfere with interpretation of study data.

15. Patients whose decision to participate might be unduly influenced by perceived expectation of gain or harm by participation, such as patients in detention due to official or legal order.

2.5 TREATMENT PLAN

2.5.1 T-Guard Treatment

T-Guard product will be supplied as 2 separate liquids for infusion, i.e.:

SPV-T3a-RTA, having a protein concentration of 0.2 mg/ml, and filled in 12.5 ml fractions in 20 RDIN (20 ml) Type I glass bottles with rubber stop and pull-off aluminum cap that are to be stored at -20°C.

WT1-RTA, having a protein concentration of 0.2 mg/ml, and filled in 12.5 ml fractions in 20 RDIN (20 ml) Type I glass bottles with rubber stop and pull-off aluminum cap that are to be stored at -20°C.

All medication used in this study will be prepared and labeled according to the rules of Good Manufacturing Practice, International Conference on Harmonization (ICH)-Good Clinical Practice (GCP) and local regulatory requirements. Since this is an open label trial, blinding procedures are not applicable.

2.5.2 Storage, Handling, and Dispensing of T-Guard

T-Guard will be prepared for infusion by the pharmacy of the participating centers.

The liquids for infusion containing either SPV-T3a-RTA or WT1-RTA are provided at a concentration of 0.2 mg protein/ml in vials containing 12.5 ml of infusion liquid in -20 °C. The vials are to be stored immediately at -20 °C freezer until use. A certificate of analysis will be provided indicating the expiry date.

Before administration to the patient, the medication is brought to room temperature for 1.5 h, mixed and transferred to a syringe. Before the start of administration, the prepared infusion syringe can be stored for up to 4 hours, at room temperature (20 ± 5 °C), in a controlled environment.

Detailed directions for use can be found in the Pharmacy Manual.

2.5.3 Drug Accountability

T-Guard will be delivered at the Investigational Pharmacy per the Pharmacy Manual. The Investigational Pharmacist at each site will be responsible for receiving, storing, distributing and accounting of the study drug. All study medication supplied for the study should be kept in a locked secure place with appropriate pharmaceutical precautions.

A “Drug Accountability” record for T-Guard should be maintained by the person responsible for dispensing the trial medication to the patient. This record should contain which supplies are issued to which patients, including the times of dosing, and any drugs returned unused. Details of any supplies that are inadvertently damaged should be reported on this record. Further information on

study drug accountability is provided in the Pharmacy Manual. Study drug accountability logs will be monitored per the clinical monitoring plan.

All unused study medication should be kept and added to the drug accountability record. All study medication in these categories will be inventoried by the monitor during and at the conclusion of the study. The monitor will arrange for their secure disposal at the end of the study.

The drugs supplied for this study are only intended for use by patients enrolled in this study. They must not be diverted for use by others.

2.5.4 Dose and Administration of T-Guard

T-Guard will be administered at a dose of 4mg/m^2 (actual body weight) over 4 hours every 2 calendar days on Days 0, 2, 4, and 6. Infusions of T-Guard that exceed 4 hours are not considered deviations. Pauses in the infusion due to a patient reaction need to be documented.

The maximum BSA to be used in dose calculation is 2.5 m^2. If the patient's BSA is more than

2.5 m^2, the dose calculation should use 2.5 m^2. The patient's weight on the day of the first dose will determine the dose for the remaining infusions (dose adjustments for changes in weight during the treatment period will not be made).

Premedication with diphenhydramine 50 mg by mouth or 25 mg IV should be administered 30 minutes prior to each dose. For pediatric patients, the diphenhydramine dose should not exceed 1 mg/kg. Premedication with acetaminophen is permitted.

It is recommended that T-Guard be infused via a central catheter using a dedicated line or can be administered via a peripheral line, if needed. If a multi-lumen central venous catheter is used, T-Guard and crystalloid or total parenteral nutrition should be administered through different lumens of the catheter. PK samples should be drawn from the central catheter using a different lumen than the lumen used for infusion.

Before the infusion, connect the syringe with the in-line filter and luer-lock extension tube to a central venous catheter (recommended) or peripheral line. The contents of the syringe will then be administered by means of an automated infusion device, over a period of 4 hours. Once the infusion syringe is empty, the administration tubes will be flushed.

Vital signs (temperature, pulse, respiratory rate, blood pressure), should be checked at the following timepoints with each infusion: just before starting the infusion, 15 minutes after the start of the infusion, 30 minutes after the start of the infusion, then every 30 minutes during the infusion, and at 1-hour post-infusion. Deviation of +/- 5 minutes may occur for all vital sign collection timepoints.

Infusion-related reactions are a potential risk with this medication. If the patient experiences a grade 3 or 4 infusion related reaction, the infusion should be held, and the reaction managed per institutional standards. Once the reaction has resolved the infusion can be restarted at half the rate. Subsequent infusions should be managed with an escalation in premedication based on type of reaction and initiated at the half rate. After 15 minutes, if no reactions are noted, then the infusion rate can be escalated to the full rate as tolerated.

Patients should receive a maximum of 4 doses. The minimal interval between doses is 2 days (no less than 40 hours apart). If required for logistics, a delay of up to 1 day can occur between one of the doses during the treatment course. If toxicity occurs, then dosing can be delayed until toxicity improves, but all dosing must be completed within 14 days.

2.5.5 Toxicities and Guidelines for Withholding T-Guard

The following are expected toxicities that may impact administration of T-Guard.

Allergic reactions:

Each of the following well-recognized allergic reactions to foreign protein may follow the administration of a monoclonal antibody including urticaria, bronchospasm, anaphylaxis, Arthus reaction, vasculitis, and serum sickness. Symptoms will be monitored closely and should be treated per SoC. Appropriate medications should be readily available at the bedside, including epinephrine, hydrocortisone and diphenhydramine.

Anaphylactic reaction:

Administration of xenogeneic proteins may be accompanied by anaphylactic reactions that are mostly IgE mediated. The most probable time of onset, if occurring, is within 10 minutes after starting T-Guard infusions. This acute hypersensitivity reactions may be characterized by: cardiovascular collapse, cardiorespiratory arrest, loss of consciousness, hypotension, pulmonary edema especially in patients with volume overload, seizures or coma, tachycardia, pruritus, urticaria, tingling, angioedema including laryngeal, pharyngeal or facial edema, dyspnea, bronchospasm, and airway obstruction.

If an anaphylactic reaction is suspected, T-Guard administration should be discontinued immediately. Therapy should not be resumed, nor should the patient be exposed to other murine immunoglobulins or RTA-containing products.

Capillary Leak Syndrome (CLS):

Manifested as hypotension, fluid overload, weight gain or edema, dyspnea, anorexia, nausea, and in some cases confusion, and muscle damage. Investigative findings, may include, hypoxia, hypoalbuminemia, pulmonary edema, pleural effusion. Patients experiencing severe CLS (requiring pressors, dialysis, mechanical ventilator support) related to study treatment should have their treatment held until 72 hours after cessation of pressors, dialysis, or mechanical ventilator support.

Hypoalbuminemia:

Severe hypoalbuminemia is a risk for CLS. Therefore, if the albumin level is less than or equal to 1 g/dL, dosing should be held until the albumin level is greater than1 g/dL. Dosing can be based on the albumin level within 48 hours of dosing. If the treating physician feels that there has been benefit from dosing, then further dosing can be discussed with the protocol chairs.

Myalgia and serum CK-elevation:

There is an association of myositis with Ricin-based immunotoxicity. Therefore, patients should be monitored for signs of myositis. If patients experience muscle pain, then a serum CK should be performed. The next dosage of T-Guard should be withheld in case of a CK elevation greater than 5 times ULN. Dosing can resume if the CK decreases to less than or equal to 5 ULN and symptoms of myositis has improved to Grade 2 or less by CTCAE v.5. Dosing can proceed at the investigator's discretion if deemed in the best interest of the patient.

Other toxicities:

Patients with another non-hematologic grade 3 or higher toxicity which is not attributable to an expected post-transplant event may have their study drug held at the attending physician's discretion. Expected post-transplant events include steroid-related toxicity and chemotherapy toxicity. T-Guard should be restarted after recovery of related toxicities to grade 2 or lower or identification of an alternative cause for these toxicities.

Infection:

Patients with severe infections resulting in hemodynamic instability requiring use of vasopressor medication may have study drug held at the discretion of the treating physician.

2.5.6 T-Guard Discontinuation

T-Guard treatment must be completed within 14 days of the first study drug dose. Patients who have missed doses may re-start T-Guard provided it is within the 14-day treatment window.

2.5.7 Acute GVHD Progression or Non-response

If additional systemic acute GVHD treatment, other than steroids, is added for lack of response or progression, T-Guard treatment must be discontinued and not re-instituted.

2.5.8 Supportive Care

2.5.8.1 Corticosteroid Dosing

For patients with SR-aGVHD a gradual taper of steroids rather than an abrupt discontinuation of steroids is recommended. Corticosteroid taper may be done per institutional practice or may follow the suggested schedule below for responding patients. Patients on 1 mg/kg/day may follow the taper beginning on week 2.

Table 2-1: Suggested Steroid Taper Protocol

Week (After study initiation)	Steroid Dose
Week 1	1.5 mg/kg/day
Week 2	1.0 mg/kg/day
Week 3	0.5 mg/kg/day
Week 4	0.25 mg/kg/day
Week 5	0.2 mg/kg/day
Week 6	0.1 mg/kg/day
Week 7	0.1 mg/kg/every other day

2.5.8.2 GVHD Prophylaxis Medications

Medications such as cyclosporine, tacrolimus, mycophenolate mofetil (MMF), sirolimus (if used as GVHD prophylaxis prior to the development of acute GVHD) may be continued, resumed, or increased to therapeutic doses per provider discretion, and adjusted/discontinued as necessary for renal, central nervous system (CNS) or other toxicity using institutional management guidelines. Resumption of a GVHD prophylaxis agent is not considered the addition of a second agent.

2.5.8.3 Topical and Ancillary GVHD Therapies

Topical treatment for acute GVHD is allowed and should be used according to institutional practices. Topical treatment, including corticosteroid creams, topical tacrolimus, oral beclomethasone or budesonide, topical azathioprine and ophthalmic glucocorticoids, is not considered as secondary systemic treatment.

Ancillary/supportive care measures for acute GVHD such as the use of anti-motility agents for diarrhea, including octreotide, is allowed at the discretion of the treating physician. Use of ursodiol to prevent/reduce gall bladder sludging or prevent hepatic transplant complications is also allowed according to institutional guidelines.

2.5.8.4 Other Supportive Care Guidelines

In addition to T-Guard, all patients should receive the following per institutional practice:

- Transfusion support
- Anti-infective prophylaxis against herpes viruses, *Pneumocystis jiroveci,* bacterial and fungal infections.
- Routine CMV antigenemia/viral load testing by hybrid capture or PCR based methods (with preemptive treatment in patients who develop a positive assay). CMV testing is recommended weekly through at least Day +100 post-transplant. Prophylaxis against CMV is allowed.
- In addition to the required monitoring for EBV according to the study calendar, monitoring for viral infections such as EBV, adenovirus, and HHV6 is encouraged for patients at high risk in accordance with institutional practice.

- Co-enrollment onto supportive care and infectious disease protocols will be allowed on case-by-case basis and requires approval by study chair or officer and sponsor.

2.5.9 Follow-Up Post Treatment Discontinuation

Patients may withdraw from treatment at any time for any reason. The reason should be documented by the study team. However, patients that have received at least one treatment dose are evaluable for all endpoints of the study and should continue with protocol-specific follow-up and data collection unless consent to study is withdrawn. Enrolled patients that do not receive any study treatment may be replaced. Patients that receive at least one treatment dose remain on study and are evaluable for all endpoints as above.

2.6 STUDY CONDUCT

This study will be conducted in accordance with the protocol, the BMT CTN 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 1802 eCRF Completion Guide. Source documentation should be made available for monitoring visits, audits and regulatory inspections as described in the BMT CTN MOP.

Participating Principal Investigators 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 ACUTE GVHD RESPONSE DEFINITIONS

Scoring of aGVHD response on a given day is in comparison to the patient's aGVHD staging on the day of initial T-Guard treatment. Organ staging will be assessed using the Harris criteria (Appendix C) and response will be scored as defined below:

Complete response (CR) is defined as a score of 0 for the GVHD staging in all evaluable organs. For example, for a response to be scored as CR, the patient must still be in CR on that day and have had no intervening additional systemic therapy for treatment of aGVHD.

Near-CR is defined as a score of 0 for the GI and/or liver GVHD staging, but with persistence of skin rash at less than 25% BSA (Stage 1 skin GVHD). Near CR can only occur in patients with both visceral and skin aGVHD.

Partial response (PR) is defined as improvement in one or more organs involved with GVHD symptoms without progression in others. For example, for a response to be scored as PR, the patient must still be in PR on that day and have had no intervening additional systemic therapy for treatment of aGVHD.

Mixed response (MR) is defined as improvement in one or more organs with deterioration in another organ manifesting symptoms of GVHD or development of symptoms of GVHD in a new organ.

No response (NR) is defined as absence of any improvement or progression as defined.

Patients receiving additional systemic therapy will be classified as non-responders.

Progression is defined as deterioration in at least one organ without any improvement in others.

Loss of CR or near-CR is defined as progression of aGVHD symptoms requiring additional systemic therapy; death; or if the patient develops new target organ symptoms that could qualify as being from either aGVHD or cGVHD (if the new symptoms are only associated with cGVHD then this is not considered a loss of CR).

3.2 RELAPSE DEFINITION

Malignancy relapse is defined as follows:

Relapse is defined by either morphological or cytogenetic evidence of acute leukemia or MDS consistent with pre-transplant features, or radiologic evidence of lymphoma, documented or not by biopsy. Progression of disease applies to patients with lymphoproliferative diseases (lymphoma or chronic lymphocytic leukemia) not in remission prior to transplantation. The event is defined as increase in size of prior sites of disease or evidence of new sites of disease, documented or not by biopsy.

Acute leukemia, CML and MDS – Relapse will be diagnosed when there is:

- Reappearance of leukemia blast cells in the peripheral blood; or,

- Greater than 5% blasts in the bone marrow, not attributable to another cause (e.g. bone marrow regeneration)
- The appearance of previous or new dysplastic changes (MDS specific) within the bone marrow with or without falling donor chimerism; or
- The development of extramedullary leukemia or leukemic cells in the cerebral spinal fluid or
- The reappearance of cytogenetic abnormalities present prior to transplantation

Lymphoproliferative Diseases – Relapse or progression will be diagnosed when there is:

- Appearance of any new lesion more than 1.5 cm in any axis during or at the end of therapy, even if other lesions are decreasing in size. Increased fluoro- deoxyglucose (FDG) uptake in a previously unaffected site will only be considered relapsed or progressive disease (PD) after confirmation with other modalities. In patients with no prior history of pulmonary lymphoma, new lung nodules identified by CT are mostly benign. Thus, a therapeutic decision should not be made solely on the basis of the positron emission tomography (PET) without histologic confirmation.
- At least a 50% increase from nadir in the sum of the product diameters of any previously involved nodes, or in a single involved node, or the size of other lesions (e.g., splenic or hepatic nodules). To be considered PD, a lymph node with a diameter of the short axis of less than 1.0 cm must increase by ≥ 50% and to a size of 1.5 x 1.5 cm or more than 1.5 cm in the long axis.
- Lesions should be PET positive if observed in a typical FDG-avid lymphoma or the lesion was PET positive before therapy unless the lesion is too small to be detected with current PET systems (less than 1.5 cm in its long axis by CT).
- In addition to the criteria above, patients with chronic lymphocytic leukemia (CLL) who present in complete remission prior to transplantation may fulfill the relapse definition if there is reappearance of circulating malignant cells that are phenotypically characteristic of CLL.

Multiple myeloma – Clinical relapse is defined as meeting one or more of the following criteria:

- Direct indicators of increasing disease and/or end organ dysfunction (CRAB features) related to the underlying clonal plasma-cell proliferative disorder. It is not used in calculation of time to progression or progression-free survival but is listed as something that can be reported optionally or for use in clinical practice;
- Development of new soft tissue plasmacytomas or bone lesions (osteoporotic fractures do not constitute progression);
- Definite increase in the size of existing plasmacytomas or bone lesions. A definite increase is defined as a 50% (and greater than or equal to1 cm) increase as measured serially by the SPD[§§] of the measurable lesion;
- Hypercalcaemia (greater than 11 mg/dL);
- Decrease in haemoglobin of greater than or equal to 2 g/dL not related to therapy or other non-myeloma-related conditions;
- Rise in serum creatinine by 2 mg/dL or more from the start of the therapy and attributable to myeloma;
- Hyperviscosity related to serum paraprotein.

For the purposes of assessing relapse free survival (RFS), if the patient is in a CR, any one or more of the following will be considered a relapse:

- Reappearance of serum or urine M-protein by immunofixation or electrophoresis;
- Development of greater than or equal to 5% plasma cells in the bone marrow;
- Appearance of any other sign of progression (i.e., new plasmacytoma, lytic bone lesion, or hypercalcaemia see above).

Myeloproliferative Neoplasms: Relapse from CR is defined as:

- Reappearance of bone marrow disease, including blasts, monocytic blast equivalents, or fibrosis
- New extramedullary disease, including new or reappearance of splenomegaly, hepatomegaly, skin lesions, etc.

***Institution of any therapy to treat persistent, progressive or relapsed malignancy, including the withdrawal of immunosuppressive therapy or donor lymphocyte infusion, will be considered evidence of relapse/progression regardless of whether the criteria described above were met.**

***Non-malignant Diseases*:** Non-malignant diseases will be considered to have a transplant status of persistent/active disease. Graft failures will be considered recurrence.

3.3 PRIMARY ENDPOINT

The primary endpoint is the proportion of patients with a CR at Day 28 after start of T-Guard treatment (first infusion).

3.4 SECONDARY ENDPOINTS

3.4.1 Key Secondary Endpoints:

3.4.1.1 Duration of Complete Response (DoCR)

DoCR is defined as the time from first observation of CR until loss of CR or the date of additional systemic therapy for treatment of aGVHD. DoCR will be evaluated both in the set of all patients who achieve a CR and only in those patients who are in CR at Day 28 post- initiation of T-Guard treatment.

3.4.1.2 Overall Survival (OS)

OS will be assessed at Days 90 and 180 post initiation of T-Guard treatment. An event for this analysis is death from any cause and time will be calculated from initiation of T-Guard treatment until date of death.

3.4.2 Other Secondary Endpoints:

3.4.2.1 Overall Response Rate (ORR)

ORR is defined as having a complete or partial response (CR+PR). The ORR will be estimated at Days 14, 28, and 56 post T-Guard initiation.

3.4.2.2 Proportion of Response

The proportion of patients in each aGVHD response category will be described at Days 7, 14, 28 and 56 post initiation of T-Guard.

3.4.2.3 Non-relapse mortality (NRM)

Events for NRM are death from any cause other than relapse/progression of the underlying malignancy. Relapse will be considered a competing risk. Time for NRM will be from time of T-Guard initiation until the earlier of death from a non-relapse cause or relapse (competing risk). NRM will be estimated at Days 100 and 180 post initiation of T-Guard.

3.4.2.4 Relapse free survival (RFS)

Events for RFS are death from any cause or relapse/progression of the underlying malignancy. Time will be calculated from the initiation of T-Guard treatment until the earlier of death or relapse/progression of the underlying malignancy. RFS will be estimated at Day 180 post initiation of T-Guard.

3.4.2.5 GVHD-free Survival

Patients alive, in CR and without cGVHD will be considered a success for this endpoint. GVHD free survival will be estimated at Days 90 and 180 post initiation of T-Guard treatment.

3.4.2.6 Chronic GVHD (cGVHD)

cGVHD is defined per NIH Consensus Criteria (see Appendix D). Time will be calculated from the initiation of T-Guard treatment until the earlier of diagnosis of cGVHD or death from any cause, with death treated as a competing risk. The cumulative incidence of cGVHD at Day 180 post T-Guard initiation will be estimated and maximum severity (mild/moderate/severe) will be described.

3.4.2.7 Relapse/Progression of Underlying Malignancy

The cumulative incidence of malignancy relapse/progression will be estimated with death prior to relapse/progression considered a competing risk. The cumulative incidence of relapse/progression at Day 180 post T-Guard initiation will be described.

3.4.2.8 Incidence of Systemic Infections

All Grade 2-3 infections (as defined by the BMT CTN MOP) from initiation of T-Guard treatment will be reported by site of disease, date of onset, and severity. Incidence of systemic infections will be described in patients from the initiation of T-Guard treatment to 28 days post last dose.

3.4.2.9 Incidence of Toxicities

All grade 3-5 toxicities according to CTCAE v5 occurring from the initiation of T-Guard treatment to 28 days post last dose will be described.

3.4.2.10 Pharmacokinetics of T-Guard

A population pharmacokinetic model will be developed for T-Guard based on the SPV-T3a- RTA and WT1-RTA levels measured in samples obtained before each infusion and at the following post-infusion timepoints: 4, 5, 6, 8, and 24 hours for the first infusion, 4, 6, and 24 hours for the

second and third infusions, and 4, 6, 24 and 48 hours for the fourth infusion. The time points for blood sampling were based on the $t_{1/2}$ and C_{max} values as determined in the previous studies. The Population PK model will be used to describe the following metrics:

- Cinf: Observed and model-predicted concentration at the end of infusion
- CL: Systemic clearance
- AUC: Model-predicted area under the curve from the start of the current infusion until the next infusion or until 48 hours following for the last infusion
- $t_{1/2}$: Model-predicted terminal half-life
- Vc: Volume of the central compartment

Additionally, the impact of various factors on these measures will be evaluated, including age, weight, BSA, BMI, disease status, and anti-drug-antibodies (ADA).

3.4.2.11 Immunogenicity of T-Guard

ADA responses in the form of human anti-SPV-T3a-RTA and anti-WT1-RTA -antibodies will be evaluated in serum samples obtained at baseline and at Days 7, 14, 28, 90, and 180 following initiation of T-Guard treatment validated bioluminescence assays.

3.5 EXPLORATORY ENDPOINTS

3.5.1 Corticosteroid Dose

The steroid dose administered (measured in prednisone equivalent) at baseline, Days 28 and 56 will be described.

3.5.1.1 Near Complete Response (Near CR)

Near CR is defined as having stage 1 skin GVHD only (persistence of skin rash at less than 25% BSA) with a score of 0 in other target organs. The proportion of patients with a near CR will be evaluated at Day 7, 14, 28, and 56 post-initiation of T-Guard treatment. The OS rates at Day 180 will also be described for patients that attain a near CR or better and those who do not.

3.5.1.2 Discontinuation of Systemic Steroids

The proportion of patients that are free of systemic steroid therapy at Day 180 post- T-Guard initiation will be described.

3.5.1.3 Incidence of CMV Reactivation

The proportion of patients requiring new systemic treatment for a CMV PCR level per institutional practice (patients receiving only SoC viral prophylaxis will not be included in this assessment) for cytomegalovirus (CMV)-reactivation by Day 180 post-T-Guard initiation will be described.

3.5.1.4 Incidence of EBV-associated Lymphoproliferative Disorder

The proportions of patients with EBV-associated lymphoproliferative disorder and EBV reactivation requiring therapy with rituximab by Day 180 post-T-Guard initiation will be described.

3.5.1.5 Incidence of IMP-related Serious Adverse Events (SAEs)

SAEs that are deemed to be related to T-guard treatment will be summarized by type, frequency, and number of patients affected.

3.5.1.6 T-cell Subsets and NK-cells

The evolution of specific cell populations over the 180-day follow-up period and, in particular, T-Guard's effect in depleting targeted T cell and NK cell subsets as well as its impact on relevant non-target populations (B cells, monocytes and dendritic cells), will be evaluated.

The Phase 1/2 study showed that when using a pan T cell marker, there is no direct correlation between the T cell depletion patterns and clinical responses. This might be explained by the observed responses being induced by a shift in the composition of the T cell compartment towards more tolerogenic subpopulations, rather than by just grossly (but temporarily) reducing the total number of circulating T cells (and NK cells).

The flow cytometry analysis as proposed below, with a more refined and detailed set of markers, might help to further elucidate T-Guard's MoA and identify a surrogated biomarker for response.

Blood samples will be taken during the infusion week at Days 0, 2, 4, 6 (just prior to and 4 hours after each T-Guard infusion) and then subsequently at Days 14, 28, 56, and 180. These samples will be assessed by to measure the following cell populations: Inflammatory Monocytes & Dendritic Cells, Recent Thymic Emigrants, CD4+, CD8+ Naïve & Memory Cells, CD4+ T Regulatory Cells, NK Cells, γδ T Cells, and B cells.

3.5.1.7 GVHD-related biomarkers

Serum ST2 and Regenerating Family Member 3 Alpha (REG3α) concentrations and urine 3-Indoxyl Sulfate (3-IS) concentrations at baseline and at Day 7, 14, and 28 post-initiation of T-Guard treatment will be used to estimate the probability of NRM at Day 180 post-assessment for each patient, using the NRM risk model from (Major-Monfried, Renteria et al. 2018). The proportion of patients with high risk biomarker status (defined as estimated NRM greater than 0.29) will be described at each time point.

3.5.1.8 Patient Reported Outcomes (PROs)

Patient reported outcomes will be assessed using a subset of the PROMIS measures described in Appendix F. PROs will be assessed at baseline and Days 28, 56, and 180 post initiation of T-Guard treatment.

3.6 ENDPOINT REVIEW PROCESS

Upon completion of patient follow-up, an Endpoint Review Committee (ERC) will conduct an independent review of site-reported data on a key study endpoint, Day 28 aGVHD response, in order to determine the data to be presented in the primary manuscript and final analysis. This Committee will consist of members of the protocol team, including the Protocol Chairs, Protocol Officer, Operational Statistician, and Protocol Coordinator. Each patient's data will be reviewed by ERC clinicians. The adjudicated Day 28 aGVHD response data for each patient will be determined by consensus of the reviewers.

Data will be obtained from the relevant electronic case report forms (eCRFs) and source documents and will be provided to reviewers in a blinded manner with respect to treatment center and patient identifier. These data will be kept confidential and will not be discussed outside the Committee or presented in a public forum. The ERC charter will provide further details on the ERC membership and adjudication process.

CHAPTER 4

4 PATIENT ENROLLMENT AND EVALUATION

4.1 APPROACHING PATIENTS, ELIGIBILITY SCREENING, AND OBTAINING CONSENT

Patients with SR-aGVHD will be approached as soon as possible after diagnosis. Patients at least 18 years of age and willing to participate in the trial will sign an Institutional Review Board (IRB) approved ICF, and eligibility for enrollment will be further evaluated. For patients at least 12 but less than 18 years of age and willing to participate, assent will be obtained in addition to parent or guardian consent. The process of obtaining informed consent must comply with applicable ICH GCP E6 guidelines as implemented in US guidelines, GCP guidelines and national regulatory requirements.

4.2 ENROLLMENT

Patients will be enrolled onto the study using Advantage eClinical. The following procedures shall be followed:

1. An authorized user at the clinical center completes the initial screening by entering patient demographics and Segment A information (consent date, inclusion/exclusion criteria) on the Eligibility Form.

2. If the patient is eligible, a patient ID number is generated.

If a connection is interrupted during an enrollment session, the process is completely canceled and logged. A backup manual enrollment system will also be available to provide for short- term system failure or unavailability.

4.2.1 Treatment]

Treatment should be initiated as soon as possible after enrollment. A maximum of 72 hours (3 days) is allowable.

4.3 STUDY MONITORING

4.3.1 Follow-up Schedule

The Follow-up Schedule for scheduled study visits is outlined in Table 4.1. The timing of follow-up visits is based on the date of first T-Guard infusion (Day 0) A detailed description of each of the forms and the procedures required for forms completion and submission can be found in the Data Management Handbook and User's Guide.

Table 4.1: Follow-up Schedule

Assessment Time	Target Day[1] (Days Post-Enrollment)
1 week	7 days
2 weeks	14 days
3 weeks	21 days

Assessment Time	Target Day[1] (Days Post-Enrollment)
4 weeks	28 days
5 weeks	35 days
6 weeks	42 days
7 weeks	49 days
8 weeks	56 days
10 weeks	70 days
90 days	90 days
6 months	180 days

[1]Target day range = ±3 days for Day 7 (subsequent visits through Day 56 must be scheduled weekly and within ±3 days of target date). Target day range ±14 days for Days 70, 90, and 180

Criteria for Forms Submission: Criteria for timeliness of submission for all study forms are detailed in the Data Management Handbook and User's Guide. Forms that are not entered in Advantage eClinical within the specified time will be considered delinquent. Transplant Centers can view past due forms via Advantage eClinical. A missing form will continue to appear until the form is entered into Advantage eClinical, or until an exception is granted and entered into the Missing Form Exception File, as detailed in the Data Management Handbook and User's Guide.

Reporting Patient Deaths: The Recipient Death Information must be entered into Advantage eClinical within 24 hours of knowledge of a patient's death even if the cause of death is unknown at that time. Once the cause of death is determined, the form must be updated.

Center for International Blood and Marrow Transplant Research (CIBMTR) Data Reporting: Centers participating in BMT CTN trials must register pre and post-transplant outcomes on all consecutive HCTs done at their institution during their time of participation to the CIBMTR. Registration is done using procedures and forms of the Stem Cell Transplant Outcomes Database (SCTOD). (Note: Federal legislation requires submission of these forms for all US allotransplant recipients.) CIBMTR post- transplant Report Forms must continue to be submitted for all patients enrolled on this trial. Long-term follow-up of patients on this study will continue through routine CIBMTR mechanisms.

4.3.2 Assessments

Assessment and/or reporting of the following is required for patients enrolled on this study. All assessments are considered standard-of-care unless identified below by "*" below. Assessments indicated by "*" are for research purposes.

Prior to Enrollment

The following pre-enrollment assessments must be completed prior to enrolling the patient in Advantage eClinical and within the designated timeframe listed below.

1. Complete acute GVHD staging and grading information including assessments of rash, diarrhea, nausea/vomiting, and bilirubin, within 24 hours prior to enrollment. Biopsy results of involved tissue, if performed as SoC, should also be reported.

2. Albumin, platelet count, and CK level within 24 hours prior to enrollment
3. Complete blood count (CBC) with differential within 24 hours prior to enrollment
4. Pregnancy test (urine or serum) for females of child-bearing potential within 30 days prior to enrollment

Baseline Assessments

Baseline assessments listed below must be completed post-enrollment and within 24 hours prior to the first dose of study therapy unless otherwise specified.

1. Karnofsky or Lansky Performance Status (may be completed pre- or post-enrollment)
2. Baseline patient-reported outcome measures (Appendix F)
3. Peripheral blood for EBV and CMV viral load (within 72 hours prior to first T-Guard dose)
4. Peripheral blood* for EBV, CMV-specific and alloreactive T-cells (See Appendix B)
5. CBC with differential and platelet count* (must be collected at the same time as the 30mL peripheral blood for alloreactive T-cells)
6. Peripheral blood* for humoral response (ADA) and immunophenotyping (See Appendix B)
7. Peripheral blood* and urine sample* for GVHD biomarker testing (See Appendix B)
8. Recording of all systemic immune suppressive therapy (as appropriate: tacrolimus, cyclosporine, etc.), as well as topical agents
9. History and physical exam including height and weight, patient disease/transplantation baseline variables (may be completed pre- or post-enrollment but must be within 7 days prior to first T-Guard dose)

At Each Infusion of T-Guard Therapy

1. Complete acute GVHD staging and grading information including assessments of rash, diarrhea, nausea/vomiting, and bilirubin
2. Vital signs (temperature, pulse, respiratory rate, blood pressure) just before starting the infusion, 15 minutes after the start of the infusion, 30 minutes after the start of the infusion, then every 30 minutes during the infusion, and at 1-hour post-infusion*. Deviation of +/- 5 minutes may occur for all vital sign collection timepoints.
3. Albumin and platelet count prior to each dose of T-Guard
4. CBC with differential
5. Peripheral blood* for immunophenotyping studies pre-infusion and at 4 hours after the start of infusion. Pre-infusion samples should be drawn prior to T-Guard administration and a window of +/- 15 minutes is allowed for the sample drawn 4 hours after the start of infusion.
6. Peripheral blood* for PK at the following timepoints
    a. 1st infusion: pre-infusion, and at 4, 5, 6, 8, and 24 hours after start of infusion
    b. 2nd infusion: pre-infusion, and at 4, 6, and 24 hours after start of infusion
    c. 3rd infusion: pre-infusion, and at 4, 6, and 24 hours after start of infusion
    d. 4th infusion: pre-infusion, and at 4, 6, 24, and 48 hours after start of infusion

Pre-infusion samples should be drawn prior to T-Guard administration. A window of +/- 15 minutes for samples drawn at 4,5, or 6 hours after the start of infusion, and a window of +/-30

minutes for the sample drawn 8 hours after the start of infusion is allowed. A window of +/- 2 hours for samples drawn 24 or 48 hours after the start of infusion is also allowed. Every effort should be made to collect PK samples at all timepoints, however, the treatment schedule should not be altered to accommodate PK sampling. For example, if T-Guard administration is scheduled for a weekend and PK sampling is not possible then administration should occur on schedule. In the event weekend PK sample collection is missed, collection of PK samples should be resumed at the next T-Guard infusion. Please refer to Section 4.5 for more information regarding infusion delays.

Post-Initiation of T-Guard Therapy

The following assessments and observations must be completed at the timepoints designated below.

1. Complete acute GVHD staging and grading information including assessments of rash, diarrhea, nausea/vomiting, and bilirubin at Days 7, 14, 21, 28, 35, 42, 49, 56, 70, 90, and 180
2. Albumin and platelet count at Days 28, 56, 90, and 180
3. CBC with differential at Days 28, 56, 90, and 180 (must be collected at the same time as the 30mL peripheral blood for alloreactive T-cell sample on Days 28 and 180)
4. Karnofsky or Lansky performance status at Days 28, 56, 90, and 180
5. Peripheral blood for EBV and CMV viral load at Days 28 and 180 with monitoring of EBV and CMV continuing per institutional practice post Day 28 through 180 days post initiation of T-Guard
6. Peripheral blood* for EBV, CMV-specific and alloreactive T-cells at Days 28 and 180
7. Patient reported outcomes at Days 28, 56 and 180 post initiation of T-Guard
8. cGVHD evaluation (if present) Day 28, 56, 90, and 180
9. Peripheral blood* for ADA testing at Days 7, 14, 28, 90, and 180
10. Peripheral blood* for immunophenotyping studies at Days 14, 28, 56, and 180
11. Peripheral blood* and urine sample* at Days 7, 14, and 28 for GVHD biomarker testing
12. Recording of all systemic immune suppressive therapy (as appropriate: tacrolimus, cyclosporine, etc.), as well as topical agents, and all anti-infectives used for prophylaxis or treatment at Days 7, 14, 21, 28, 35, 42, 49, 56, 70, 90, and 180
13. Recording of toxicities on Days 56, 70, 90, and 180
14. Recording of AE/SAEs as described in Section 4.4 Adverse Event Reporting
15. Recording of infections through Day 180
16. Recording of primary disease relapse through Day 180

Table 4.2: Assessment Schedule

	Pre-Enrollment	Baseline[1]	Days of T-Guard Infusion				Days Post Initiation of T-Guard										
	Baseline		**0**	**2**	**4**	**6**	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**	**70**	**90**	**180**
Acute GVHD evaluation, including bilirubin	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Chronic GVHD evaluation										X				X		X	X
Recording of all systemic immune suppression, including steroid dose		X					X	X	X	X	X	X	X	X	X	X	X
History and physical exam		X															
CBC with differential		X[3]	X	X	X	X				X[3]				X		X	X[3]
Platelet count	X	X[3]	X	X	X	X				X[3]				X		X	X[3]
Albumin	X		X	X	X	X				X				X		X	X
CK levels[2]	X																
Pregnancy test (if applicable)	X																
Karnofsky/Lansky performance status		X								X				X		X	X

	Pre-Enrollment	Baseline[1]	Days of T-Guard Infusion				Days Post Initiation of T-Guard										
	Baseline		**0**	**2**	**4**	**6**	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**	**70**	**90**	**180**
Peripheral blood for CMV and EBV viral load		X								X							X
Peripheral blood for EBV, CMV-specific and alloreactive T-cells[3]		X								X							X
Pharmacokinetics[4]			X	X	X	X											
Peripheral blood for ADA		X					X	X		X						X	X
Peripheral blood for GVHD biomarkers REG3α and ST2		X					X	X		X							
Peripheral blood for immunophenotyping[5]		X	X	X	X	X		X		X				X			X
Urine sample for GVHD biomarker 3-IS		X					X	X		X							
Vital signs (temperature, pulse, respiratory rate, blood pressure)[6]			X	X	X	X											
AE/SAE assessment[7]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Toxicity assessment														X	X	X	X
Patient-reported outcomes: PROMIS		X								X				X			X
Infection monitoring	Reporting of infections at time of occurrence from time of enrollment through Day 180																
Primary disease relapse reporting	Reporting of relapse at time of occurrence from time of enrollment through Day 180																

[1]Baseline assessments must be completed post enrollment and within 24 hours prior to the first dose of study treatment unless otherwise specified.
[2]If patients experience muscle pain, then a serum CK should be performed.
[3]The CBC with differential and platelet count must be taken at the same time as the peripheral blood is drawn for EBV, CMV-specific and alloreactive T-cells at baseline, day 28, and day 180.
[4]Pharmcokinetic blood samples directly pre-infusion, and at 4, 5, 6, 8, and 24 hours after start of 1st infusion, pre-infusion, and at 4, 6, and 24 hours after the 2nd and 3rd infusion, and pre-infusion, and at 4, 6, 24 and 48 hours after the 4th T-Guard infusion.
Pre-infusion samples should be drawn prior to T-Guard administration. A window of +/- 15 minutes for samples drawn at 4,5, or 6 hours after the start of infusion, and a window of +/-30 minutes for the sample drawn 8 hours after the start of infusion is allowed. A window of +/- 2 hours for samples drawn 24 or 48 hours after the start of infusion is also allowed. Every effort should be made to collect PK samples at all timepoints, however, the treatment schedule should not be altered to accommodate PK sampling. For example, if T-Guard administration is scheduled for a weekend and PK

sampling is not possible then administration should occur on schedule. In the event weekend PK sample collection is missed, collection of PK samples should be resumed at the next T- Guard infusion.
[5]Peripheral blood for immunophenotyping should be collected pre-infusion and at 4 hours after the start of each infusion. Pre-infusion samples should be drawn prior to T-Guard administration and a window of +/- 15 minutes is allowed for the sample drawn 4 hours after the start of infusion.
[6]Vital signs should be taken directly pre-infusion, 15 minutes after the start of the infusion, 30 minutes after the start of the infusion, then every 30 minutes during the infusion, and at 1-hour post-infusion. Deviation of +/- 5 minutes may occur for all vital sign collection timepoints.
[7] AEs and SAEs will be reported as described in Section 4.4 Adverse Event Reporting

4.4 ADVERSE EVENT REPORTING

AE reporting requirements are summarized below.

4.4.1 Definitions

Adverse Event: An AE is any untoward medical occurrence in a patient administered an investigational product or has undergone study procedures and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease that is temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

An abnormality identified during a medical test (e.g., laboratory parameter, vital sign, ECG data, physical exam) should be defined as an AE only if the abnormality meets 1 of the following criteria:

- Induces clinical signs or symptoms.
- Requires active intervention.
- Requires interruption or discontinuation of study medication.
- The abnormality or investigational value is clinically significant in the opinion of the investigator.

Serious Adverse Event: An 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 patient 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 patient 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 an SAE). Any event is considered a SAE if it is associated with clinical signs or symptoms judged by the investigator to have a significant clinical impact.

An adverse event can be Anticipated or Unanticipated:

- **Anticipated adverse events** are those that have been previously identified as resulting from the underlying disease, the HCT, or acute GVHD and not related to study drug.
- **Unanticipated adverse events** are those that vary in nature, intensity or frequency from information in the current anticipated event list, the Investigator's Brochure, the package insert, or when it is not included in the informed consent document as a potential risk. Unanticipated events would also include those that have not been previously described as a result of the underlying disease requiring HCT, the HCT or acute GVHD

4.4.2 Classification of Adverse Events by Severity

The severity refers to the intensity of the reported event. The Investigator must categorize the severity of each unanticipated SAE according to the NCI CTCAE version 5.0. CTCAE guidelines can be referenced at the following website: http://ctep.cancer.gov/reporting/ctc.html. 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

4.4.3 Classification of Adverse Events by Relationship to Investigational Product

The relationship of each reported event to the study treatment 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 treatment interventions and dechallenge or rechallenge according to the following guidelines:

- **Possibly, Probably, or Definitely Related:** there is a reasonable possibility that the study treatment 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 not considered related for the purposes of regulatory authority reporting. One or more of the following criteria apply:

4.4.4 Required Adverse Event Reporting

Any unanticipated SAEs from time of enrollment through the study defined follow up are required to be reported following the BMT CTN Administrative MOP Chapter 6. Additionally, any grade 4 anticipated event not collected on the calendar-driven toxicity or specified event- driven form must **also** be reported through the expedited AE reporting system in Advantage eClinical. Chapter 6 of the BMT CTN MOP defines reporting based on the terms Unexpected and Expected; however, for this study, the reporting will be based on the terms Unanticipated and Anticipated All adverse events, including SAEs, must be reported from the 1st dose of T- Guard through 30 days after the last dose. In addition, any SAEs occurring after that 30-day period, but assessed as related to the investigational product, must be reported. All SAEs are to be followed up until resolved, judged to be no longer clinically significant, or until they become chronic to the extent that they can be fully characterized.

Infections are collected separately in eClinical as this is a study endpoint; however, should an infection meet the SAE criteria the infection must also be reported following the SAE reporting criteria outlined above.

GVHD and underlying disease relapse events are also collected separately in eClinical because they are part of the endpoint analysis. Events of GVHD and underlying disease relapse are not to be reported as AEs/SAEs for this study.

The Sponsor has a list of events classified as Special Situations. These events are 1) medication error, 2) overdose and 3) pregnancy. These events must be reported following the reporting for SAEs.

SAEs that require reporting will be reported through an expedited AE reporting system via an EDC**. All SAEs, as well as Special Situations, must be reported within 24 hours of knowledge of the event.** Events entered in EDC will be reported using NCI's CTCAE Version 5.0. If there are network outages, a paper copy of the AE form must be completed and emailed to Emmes to initiate Sponsor review. Once the system is available, the event information must be entered into the system.

Anticipated AEs will be reported using NCI's CTCAE Version 5.0 at regular intervals as defined on the Form Submission Schedule, including calendar-driven eCRFs (e.g., Toxicity and GVHD) or event-driven eCRFs (e.g., Relapse/Progression, Infection, Secondary Graft Failure and Death).

The Data and Safety Monitoring Board (DSMB) will receive reports of all unanticipated and unexpected SAEs upon review by the BMT CTN Medical Monitor and the Xenikos Medical Monitor. Summary reports for all reported SAEs will be reviewed by the DSMB on a semi- annual basis.

4.4.5 Procedure in Case of Pregnancy

If a female patient becomes pregnant during the study dosing period or within 90 days from the last dose of study drug, the investigator should report the information 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. If a patient becomes pregnant during the study dosing period, the investigational product will be discontinued.

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 30 days after birth should be reported as an SAE regardless of its relationship with the study drug
- If an infant dies more than 30 days after 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 180 and 360 days after birth.

4.5 INFUSION DELAYS

The visit schedule in eClinical is set up to allow patients a timeline of two days between each infusion visit and a +1-day window for logistics. In the event dosing must be delayed due to occurrence of toxicity, the protocol allows up to 14 days to receive all four infusions. Therefore, instances may occur where a visit falls outside the windows in eClinical that overlap with the visit window for the Day 7 and Day 14 visits. If the infusion visits are delayed, then the assessments for each infusion should still be done and entered into the corresponding visits' forms. Note that the Day 2, 4, and 6 visits directly correlate to Infusion #2, 3, and 4.

For example, if the third infusion (Day 4) is received five days after the second infusion (Day 2), then all the assessments scheduled for the third infusion must be completed. Data for the third infusion would still be entered on the Day 4 forms even though the third infusion occurred on a later day. Similarly, the data for the fourth infusion (Day 6) would be entered on the Day 6 forms although the fourth infusion occurred on a later day.

If an infusion is delayed and occurs on Day 7, then the infusion day assessments must be done while the Day 7 assessments can either be done on the same day or on another day within window, per PI discretion. If all assessments for the infusion (Day 4 or 6) and Day 7 are combined to be completed on the same day, the following should occur:

- Acute GVHD form and Acute GVHD Medications form should be collected and entered in eClinical for the Day 7 visit. A missing form exception should be requested for the Acute GVHD assessment for the infusion visit forms (Day 4 or 6). When requesting a missing form exception, select "Other" as the reason for the missing form exception and specify "Form no longer required, combined with Day [XX] visit".
- The specimen acquisition forms should be completed for both the Day 7 visit and the infusion visit forms (Day 4 or 6) as different samples are required.
    - Day 7: Peripheral blood for ADA, Peripheral blood for GVHD biomarkers REG3α and ST2, and a urine sample for GVHD biomarker 3-IS are to be collected prior to infusion. If the samples can't be drawn pre-infusion, they may be collected the day

after.

- Infusion Visit: CBC with differential, platelet count with differential, and albumin, pharmacokinetics, and Peripheral blood for immunophenotyping are to be collected per protocol.

- The above would still apply if an infusion was delayed to Day 8-10 and the Day 7 visit had not yet been completed.

If an infusion is delayed and occurs on Day 14, then the infusion day assessments must be done while the Day 14 assessments can either be done on the same day or on another day within window, per PI discretion. If all assessments for the infusion (Day 4 or 6) and Day 14 are combined to be completed on the same day, the following should occur:

- Acute GVHD form and Acute GVHD Medications form should be collected and entered in eClinical for the Day 14 visit. A missing form exception should be requested for the Acute GVHD assessment for the infusion visit forms (Day 4 or 6). When requesting a missing form exception, select “Other” as the reason for the missing form exception and specify “Form no longer required, combined with Day [XX] visit”.

- The specimen acquisition forms should be completed for both the Day 14 visit and the infusion visit forms (Day 4 or 6) as different samples are required.

  - Day 14: Peripheral blood for ADA, Peripheral blood for GVHD biomarkers REG3α and ST2, and a urine sample for GVHD biomarker 3-IS are to be collected prior to infusion. If the samples can’t be drawn pre-infusion, they may be collected the day after.

  - Infusion Visit: CBC with differential, platelet count with differential, and albumin, and pharmacokinetics per protocol

  - Both visits: Peripheral blood for immunophenotyping – this should be entered under the infusion visit as it is to be collected pre-infusion and 4 hours post

  - If an infusion is delayed to Day 11-13, the Day 14 visit can be combined as appropriate.

Note:

- There is a 3-day window for the Day 7 and Day 14 visits and visits should be combined when appropriate. However, it’s ideal for the assessments to be completed on Day 7 and Day 14.

- Sites are to be mindful of the max blood draw volumes per day for participants and to combine sample collection to avoid unnecessary needle sticks.

- There should be at least 4 but preferably 7 days between the biomarker samples.

CHAPTER 5

5 STATISTICAL CONSIDERATIONS

5.1 STUDY DESIGN AND OBJECTIVE

This study is designed as an open-label, single arm, non-comparative, multi-center Phase III study to investigate the efficacy and safety of T-Guard in adult and adolescent patients with SR-aGVHD. A total of 47 patients will be enrolled from approximately 20 transplant centers and treated with T-Guard. The primary objective is to assess the Day 28 CR rate with all patients followed through Day 180 to assess longer term secondary outcomes. As this is a single arm open label study, the analysis will be descriptive, with the target sample size based on ruling out CR rates based on historical data in comparable populations. The target enrollment is 47 patients treated with at least one T-Guard infusion. If any enrolled patients do not initiate T-Guard treatment, additional patients will be enrolled until the goal of 47 treated patients is met. The basis for the sample size, including the underlying meta-analysis, is described in the sequel.

5.1.1 Accrual and Study Duration

Accrual is estimated to require 365 days to complete with a total study duration of 545 days.

5.1.2 Randomization and Blinding

No randomization or blinding will occur as this is a single arm open label trial.

5.1.3 Analysis Populations

The primary analysis population will consist of all patients who are enrolled and initiate T- Guard treatment. Patients who are enrolled but do not initiate treatment of T-Guard will be described separately.

A PK analysis population will be defined for the assessment of pharmacokinetic and immunogenicity endpoints. This will consist of the set of patients in the primary analysis population from whom samples have been collected and evaluated for these endpoints.

A secondary, supportive analysis of a per protocol (PP) population is planned. The PP population will consist of all enrolled patients who:

- Received four T-Guard infusions
- Met all inclusion and exclusion criteria, with the exceptions of the use of contraceptive and the informed consent (provided a prospective ICF can be obtained)

5.1.4 Primary Hypothesis

The primary endpoint is the Day 28 CR rate among all patients. Historical control data indicates a Day 28 CR rate of 25-30% is expected under currently available treatment strategies for SR-aGVHD. The primary hypothesis is that the Day 28 CR rate under T-Guard treatment will be superior to historical rates. This will be evaluated by comparing the null hypothesis that the Day 28 CR rate under T-Guard treatment is 30% to the alternative hypothesis that this rate exceeds 30% using a one-sided test at a significance level of 2.5%.

5.1.5 Sample Size and Power Considerations

Based on the results of the Phase I/II study (Groth, van Groningen et al. 2018), we expect a Day 28 CR rate of 50% under T-Guard treatment. A literature review suggests a Day 28 CR rate of 25-30% in historical controls, summarized below:

- A random-effects meta-analysis of the 11 prospective studies on second line treatment for aGVHD reported in Martin et al. 2012 (see Figure 5-1) resulted in a CR rate of 29.5% (95% CI 24.0 – 35.3%). However, in seven of the 11 studies CRs that were achieved after Day 28 were included, and one study used CR assessment shorter than 28 days. In addition, eight of the 11 studies did not specify whether addition of another therapy to the protocol-defined treatments would result in classification of response as a non-responder.
- The recently published REACH1 study (Jagasia, Zeiser et al. 2018) with the JAK 1/2 inhibitor ruxolitinib (Jakafi/Jakavi) resulted in a CR rate at Day 28 of 26.8%.
- A meta-analysis of the 20 T-Guard treated patients of the Phase I/II study, 42 institutional control patients from that study's two centers, 77 matched control patients from the MAGIC database, and 71 patients from the REACH1 study resulted in Day 28 CR rates of 49.6% (95% CI 28.8 – 70.4%) in the T-Guard patients and 23.0% (95% CI 17.2 – 28.9%) in the control patients, adjusted for Harris acute GVHD grade (Table 5-1). CR rates were adjusted at the same ratio as observed in the T-Guard arm (3:10:7 for grades II:III:IV) using a generalized linear model adjusted for Harris grade and data source (Phase I/II, institutional controls / MAGIC / REACH1).

Figure 5-1: Forest Plot of CR Rates from the Meta-analysis on Second Line aGVHD Therapy from (Martin, Rizzo et al. 2012) [numbers next to study treatment name refer to the references in that paper].

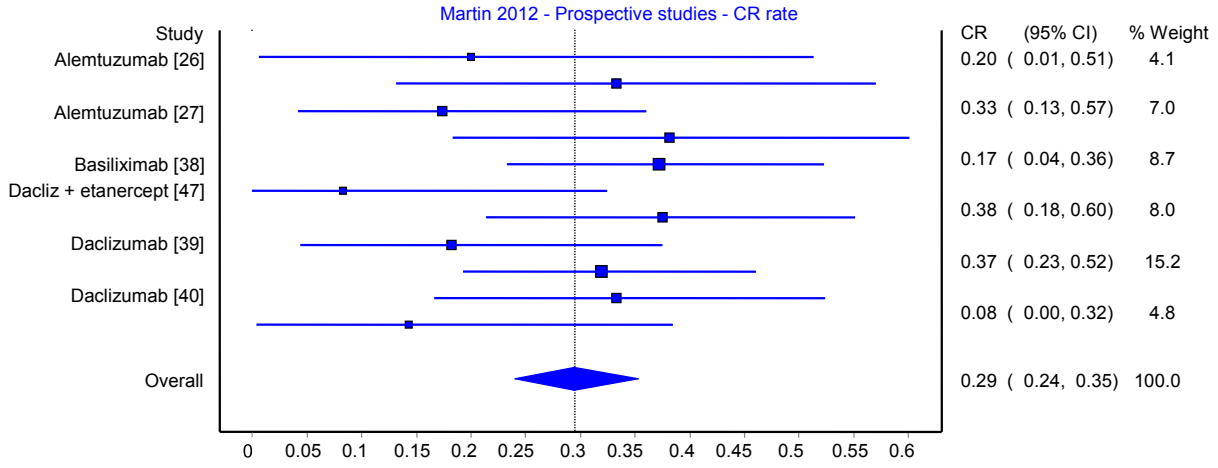


Table 5-1: Observed and Adjusted Day 28 CR Rates from Meta-analysis of Phase I/II Study, MAGIC Controls, and REACH1 Study

Harris Grade	Phase I/II T-Guard arm	Combined controls		Institutional controls	MAGIC controls	REACH1 study
II	2/3 (66.7%)	17/35 (48.6%)		-	6/12 (50.0%)	11/23 (47.8%)
III	5/10 (50.0%)	19/88 (21.6%)		3/20 (15.0%)	9/34 (26.5%)	7/34 (20.6%)
IV	3/7 (42.9%)	10/67 (14.9%)		5/22 (22.7%)	4/31 (12.9%)	1/14 (7.1%)
All	10/20 (50.0%)	46/190 (24.2%)		8/42 (19.0%)	19/77 (24.7%)	19/71 (26.8%)
Adjusted CR Rate (95% CI)	49.6% (28.8 – 70.4%)	23.0% (17.2 – 28.9%)		25.7% (13.2 – 38.2%)	20.2% (10.5 – 29.8%)	24.6% (15.4 – 33.8%)

The primary hypothesis will be evaluated by comparing the proportion of patients attaining a Day 28 CR to a historical rate of 30%, targeting 80% power to see a CR rate of 50% under T-Guard treatment. In addition, one interim analysis for futility will be incorporated. To generate the required sample size and futility and efficacy boundaries for this test, we used a Simon's two-stage minimax design. A sample size of 47 patients is required under this design to compare a null rate of 30% to an alternative rate of 50% with a one-sided type I error rate of 2.5% and 80% power.

5.2 INTERIM ANALYSIS AND STOPPING GUIDELINES

5.2.1 Interim Analysis for Futility

This trial will include one interim analysis for futility. At the interim analysis, the number of Day 28 CRs will be compared to a futility boundary determined by Simon's two-stage minimax design comparing a null rate of 30% to an alternative rate of 50% with a one-sided type I error rate of 2.5% and 80% power. This is a non-binding stopping rule, in that the type I error rate will be controlled at 2.5% even if the rule is not followed exactly. If the number of CRs fails to exceed the boundary, the DSMB will be consulted on the appropriate course of action, including whether to declare futility and close the trial early.

The futility boundaries and operating characteristics of this design are displayed in Table 5- 2A. The interim analysis will be conducted when 21 patients are evaluable for Day 28 CR. If the number of CRs is 6 or below, the boundary is crossed. At the final analysis, the futility boundary is 20 CRs and efficacy is declared only if the observed number of CRs exceeds this boundary, i.e. the observed rate is at least 21/47 (44.6%).

Table 5-2A: Single Boundary Non-Binding Futility Stopping Thresholds from Simon's Two-stage Minimax Design with One-Sided Type I Error 2.5% and Power 80%

Analysis	Number of Evaluable Patients	Futility Boundary - # of CRs (CR rate)	Probability of Stopping Early Under True CR Rate Below	
			30%	50%
Interim	21	6 (28.5%)	55.1%	3.9%
Final	47	20 (42.6%)	-	-

5.2.2 Guidelines for Safety Monitoring

Monitoring of key safety endpoints will be conducted monthly, and if their rates significantly exceed pre-set thresholds, the NHLBI will be notified in order that the DSMB can be advised. Policies and composition of the DSMB are described in the BMT CTN's Manual of Procedures. These stopping guidelines serve as triggers for consultation with the DSMB for additional review and are not formal "stopping rules" that mandate automatic closure of study enrollment. The number of enrolled patients that fail to receive any T-Guard treatment and the occurrence of toxicity, adverse events, and other safety endpoints will be monitored regularly. These data will be reported to the DSMB at semi-annual meetings at a minimum; in the event that any safety concerns arise, these data will be conveyed to the DSMB expeditiously. Additionally, the DSMB will conduct a full review of study endpoint and safety data after 10 and 30 participants are evaluable for Day 28 CR.

One key safety endpoint for this study is Day 30 overall mortality. The rate of mortality within 30 days following T-Guard initiation will be monitored in patients who initiate this treatment. Based on historical data from MAGIC, the expected probability of Day 30 mortality in SR- aGVHD patients after initiating second line treatment is 15%, while a rate of 30% is considered to be unacceptable. A sequential probability ratio test (SPRT) for binary data that compares a rate of 15% under the null hypothesis to a rate of 30% under the alternative hypothesis will be used for monthly monitoring of the Day 30 overall mortality rate in this study.

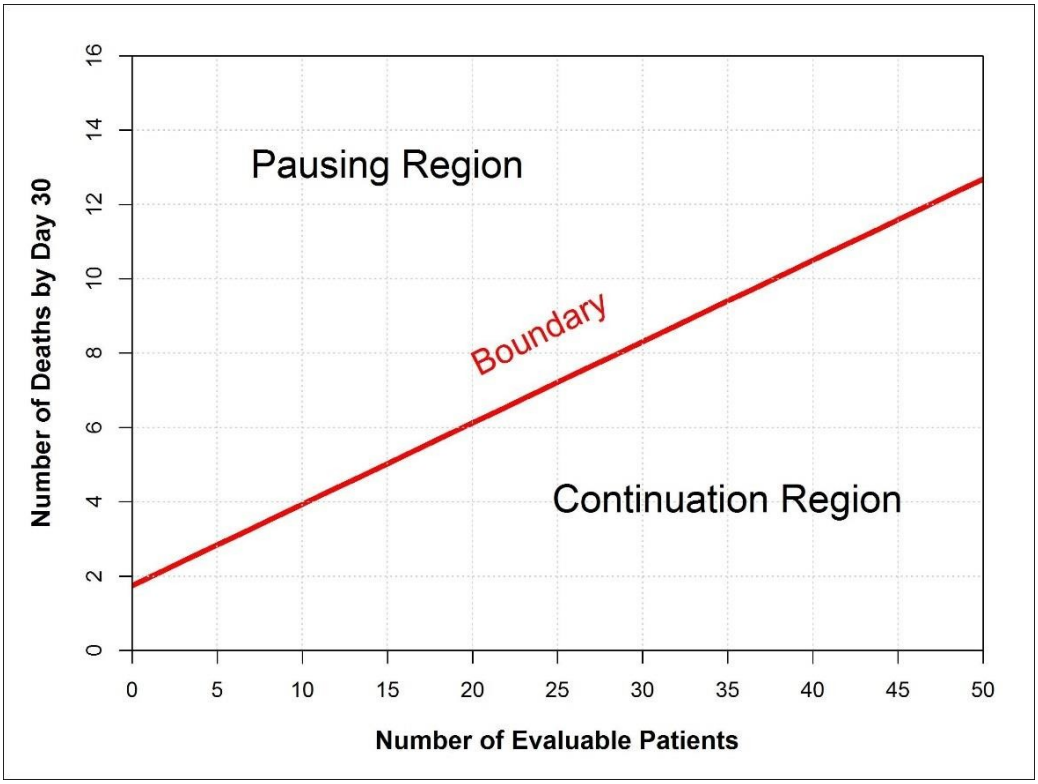
This sequential testing procedure preserves the type I error rate at a prespecified level across all of the monthly examinations. The binary SPRT can be represented graphically. At each month, the number of evaluable patients is plotted against the cumulative number of events.

The continuation region of the SPRT is defined by two parallel lines. Only the upper boundary will be used for monitoring in order to protect against excessive Day 30 mortality. If the cumulative number of deaths falls above the upper boundary the SPRT rejects the null hypothesis and concludes that more deaths occurred than should be expected in the observed number of evaluable patients. Otherwise, the SPRT continues until enrollment reaches the target sample size of 47 patients. The SPRT can be displayed in a tabular form, as shown in Table 5-2B. This table gives the rejection boundaries for the number of Day 30 mortality events corresponding to the number of evaluable patients. At least three deaths must be observed in order to trigger review. Figure 5-2A displays the same information in a graphical format.

Table 5-2B: Sequential Monitoring Plan of Day 30 Overall Mortality in 47 Patients

Number of Evaluable Patients	Rejection Boundary for Number of Day 30 Deaths
1-2	
3-5	3
6-10	4
11-14	5
15-19	6
20-24	7
25-28	8
29-33	9
34-37	10
38-42	11
43-46	12
47	13

Figure 5-2A: Rejection Boundary for Day 30 Overall Mortality Monitoring in 47 Patients



The actual operating characteristics of the truncated test are shown in Table 5-2C, obtained from a simulation study that assumed uniform accrual of 47 patients over a 365-day period with 395 days of monitoring.

Table 5-2C: Operating Characteristics of the Binary SPRT for Day 30 Overall Mortality from a Simulation Study with 100,000 Replicates

True Day 30 Mortality Rate	15%	20%	25%	30%
Probability Reject Null	0.096	0.299	0.579	0.814
Mean Month Stopped	12.3	11.0	9.2	7.3
Mean # Deaths by Day 30	6.6	7.9	8.1	7.4
Mean # Patients Enrolled	44.7	40.5	34.4	27.9

The testing procedure rejects the null hypothesis in favor of the alternative 9.6% of the time when the true Day 30 mortality rate is 15% and 81.4% of the time when the rate is 30%. This corresponds to a type I error rate of 9.6% and a type II error rate of 18.6%. If the true Day 30 mortality rate is 30%, the DSMB will be consulted 220 days after opening on average, when 7 events have been observed in 28 patients.

CTCAE Grade 4 or higher capillary leak syndrome (CLS) is another key safety endpoint that will be monitored during this study. The rate of Grade 4 or higher CLS occurring within 30 days following T-Guard initiation will be monitored in patients who initiate this treatment. This monitoring will compare a null rate of 5% to an alternative rate of 15%, considered to be unacceptable, using a sequential probability ratio test (SPRT) for binary data. The SPRT can be displayed in a tabular form, as shown in Table 5-2D. This table gives the rejection boundaries for the number of Day 30 CLS events corresponding to the number of evaluable patients. At least two events must be observed in order to trigger review. Figure 5-2B displays the same information in a graphical format.

Table 5-2D: Sequential Monitoring Plan of Day 30 Grade 4 or Higher Capillary Leak Syndrome in 47 Patients

Number of Evaluable Patients	Rejection Boundary for Number of Day 30 Events
1	
2-7	2
8-18	3
19-29	4
30-39	5
40-47	6

The actual operating characteristics of the truncated test are shown in Table 5-2E, obtained from a simulation study that assumed uniform accrual of 47 patients over a 365-day period with 395 days of monitoring.

Table 5-2E: Operating Characteristics of the Binary SPRT for Day 30 Grade 4 or Higher Capillary Leak Syndrome from a Simulation Study with 100,000 Replicates

True Day 30 Grade 4 or Higher CLS Rate	5%	10%	15%	20%
Probability Reject Null	0.094	0.466	0.807	0.954
Mean Month Stopped	12.3	9.9	7.3	5.4
Mean # CLS events by Day 30	2.2	3.5	3.7	3.5
Mean # Patients Enrolled	44.7	36.8	27.8	21.1

The testing procedure rejects the null hypothesis in favor of the alternative 9.4% of the time when the true Day 30 Grade 4 or higher CLS rate is 5% and 80.7% of the time when the rate is 15%. This corresponds to a type I error rate of 9.7% and a type II error rate of 19.3%. If the true Day 30 Grade 4 or higher CLS rate is 15%, the DSMB will be consulted 220 days after opening on average, when 4 events have been observed in 28 patients.

5.2.3 Demographic and Baseline Characteristics

Demographic and baseline characteristics will be summarized for all patients. Characteristics to be examined are: age, gender, race/ethnicity, performance status, primary disease, primary disease status, disease risk index (DRI), time from steroid initiation to enrollment, acute GVHD grade and organ staging, graft source, GVHD prophylaxis, conditioning regimen, time from disease diagnosis to transplant, time from transplant to enrollment, and time from acute GVHD onset to enrollment.

Counts and percentages will be used to describe categorical variables, while the median, mean, standard deviation, and range will be used to summarize continuous variables.

5.3 ANALYSIS OF PRIMARY ENDPOINT

The primary endpoint of this trial is the proportion of patients that attain a Day 28 CR. Patients in a CR at Day 28 post-initiation of T-Guard treatment will be considered a success while all other patients will be considered a failure for this endpoint. The CR rate at Day 28 will be estimated in the primary analysis population (all patients that initiate T-Guard treatment) using the sample proportion and a 95% Wilson score or Clopper-Pearson CI, where appropriate.

The primary determination of efficacy of T-Guard treatment on Day 28 CR will be evaluated using efficacy and futility boundaries from the Simon's two-stage minimax design described in Section 5.2.1.

5.4 ANALYSIS OF SECONDARY ENDPOINTS

All secondary endpoints will be reported for the primary and PP analysis groups.

Key Secondary Endpoints

5.4.1 Duration of Complete Response (DoCR)

DoCR is defined in Section 3.4. DoCR will be evaluated both in the set of all patients who achieve a CR and only in those patients who are in CR at Day 28 post-initiation of T-Guard

treatment. Estimates and 95% CIs for the median and quartiles of DoCR will be obtained using the Kaplan-Meier estimator.

5.4.2 Overall Survival (OS)

OS is defined as survival of death from any cause. The time from initiation of T-Guard treatment until death from any cause will be described using the Kaplan-Meier estimator. An estimate and 95% CI for OS at Days 90 and 180 post-initiation of T-Guard will be provided.

Other Secondary Endpoints:

5.4.3 Overall Response Rate (ORR)

Overall response is defined as either a complete or patient response (CR+PR). The ORR will be estimated at Days 14, 28, and 56 post T-Guard initiation using sample proportions and 95% Wilson score or Clopper-Pearson CIs, as appropriate.

5.4.4 Proportion of Response

The proportion of patients in each aGVHD response category (CR, PR, MR, NR, or progression) will be described at Days 7, 14, 28, and 56 using sample proportions.

5.4.5 Non-relapse Mortality (NRM)

NRM is defined as death from any cause other than malignancy relapse/progression. The time from initiation of T-Guard until NRM will be described using the Aalen-Johansen estimator, with malignancy relapse/progression treated as a competing risk. Estimates and 95% CIs of the cumulative incidence of NRM will be provided at Days 100 and 180 post- initiation of T-Guard.

5.4.6 Relapse-free Survival (RFS)

RFS is defined as being alive and free of malignancy relapse/progression. The time from initiation of T-Guard until malignancy relapse/progression or death will be described using the Kaplan-Meier estimator. An estimate and 95% CI for RFS at Day 180 post-initiation of T- Guard will be provided.

5.4.7 GVHD-free Survival

GVHD-free survival is defined as being alive, in CR, and free of cGVHD. The proportion of patients with GVHD-free survival at Days 90 and 180 post-initiation of T-Guard will be estimated using the sample proportion and 95% Wilson score or Clopper-Pearson CIs, as appropriate, if no censoring occurs prior to Day 180. If censoring does occur, Days 90 and 180 GVHD-free survival will be assessed using the Aalen-Johansen estimator in an illness- death multi-state model.

5.4.8 Chronic GVHD (cGVHD)

cGVHD severity is defined per the 2014 NIH Consensus Criteria (see Appendix D). The time from initiation of T-Guard treatment until onset of cGVHD of any severity (mild, moderate, or severe) will be described using the Aalen-Johansen estimator, with death prior to cGVHD onset treated as a competing risk. An estimate and 95% CI of the cumulative incidence of cGVHD will be provided at Day 180 post-initiation of T-Guard. The maximum severity of cGVHD through Day 180 post-initiation of T-Guard will be tabulated.

5.4.9 Relapse/progression of the Underlying Malignancy

The time from initiation of T-Guard until malignancy relapse/progression will be described using the Aalen-Johansen estimator, with death prior to relapse/progression treated as a competing risk. An estimate and 95% CI of the cumulative incidence of malignancy relapse/progression will be provided at Day 180 post-initiation of T-Guard.

5.4.10 Incidence of Systemic Infections

The frequency of Grade 2-3 infections occurring from the initiation of T-Guard treatment until 28 days post-last dose will be tabulated by disease site, date of onset, and severity, with Grade defined per the BMT CTN Technical MOP. The cumulative incidence of Grade 2-3 infections at 28 days post-last dose will be described using the Aalen-Johansen estimator and its 95% CI, with death prior to infection treated as a competing risk.

5.4.11 Incidence of Toxicities

The frequency of Grade 3-5 toxicities per CTCAE version 5 occurring from the initiation of T-Guard treatment until 28 days post-last dose will be tabulated by organ system. The maximum severity of reported toxicities during that period will also be summarized.

5.4.12 Pharmacokinetics

A population pharmacokinetic model will be developed for T-Guard based on the SPV-T3a- RTA and WT1-RTA levels measured in samples obtained before each infusion and at pre- defined timepoints after infusion (Table 4.2). The model will be used to evaluate the following metrics:

- Cinf: Observed and model-predicted concentration at the end of infusion
- CL: Systemic clearance
- AUC: Model-predicted area under the curve from the start of the current infusion until the next infusion or until 48 hours following for the last infusion
- $t_{1/2}$: Model-predicted terminal half-life
- Vc: Volume of the central compartment

Additionally, the impact of various factors on these measures will be evaluated, including age, weight, BSA, BMI, disease status, and ADA.

5.4.13 Immunogenicity

ADA responses in the form of anti-SPV-T3a-RTA and anti-WT1-RTA antibodies will be evaluated using serum samples obtained at baseline and at Days 7, 14, 28, 90, and 180 following initiation of T-Guard treatment. Antibody levels will be described using descriptive statistics at each time point. Changes in levels from baseline will be evaluated using Wilcoxon signed rank tests.

5.4.14 Subgroup Analyses of Day 28 CR Rate

In a secondary analysis of the primary endpoint, the CR rate at Day 28 will be evaluated separately by age group (adulthood vs. adolescence), aGVHD grade, and DRI. Point estimates and 95% Wilson score or Clopper-Pearson CIs will be provided for each subgroup, as appropriate.

5.5 ANALYSIS OF EXPLORATORY ENDPOINTS

The analysis of exploratory endpoints will be based on the primary analysis population.

5.5.1 Corticosteroid Dose

The current steroid dose being administered (measured in prednisone equivalent) will be described by mean, median, range, and quartiles at baseline and Day 28 and 56 post T-Guard initiation.

5.5.2 Near Complete Response (Near CR)

The proportion of patients with a near CR (having isolated stage 1 skin GVHD) will be estimated at Day 7, 14, 28, and 56 using sample proportions and 95% Wilson score or Clopper-Pearson CIs, where appropriate.

Estimates and 95% CIs of OS at Day 180 post-initiation of T-Guard treatment will also be obtained from the Kaplan-Meier estimator separately for two subsets of patients: those that attain a near CR or CR, and those who fail to reach a (near) CR.

5.5.3 Discontinuation of Systemic Steroids

The proportion of patients that are free of systemic steroids above physiological replacement at Day 180 post-T-Guard initiation will be described.

5.5.4 CMV Reactivation

Among patients that were CMV positive at enrollment, the cumulative incidence of initiation of systemic treatment for CMV-reactivation will be described using the Aalen-Johansen estimator, with death treated as a competing risk. An estimate and 95% CIs of the cumulative incidence of CMV reactivation will be provided at Day 180 post-initiation of T-Guard.

5.5.5 EBV-associated Lymphoproliferative Disorder

The cumulative incidence of either EBV-associated lymphoproliferative disorder or EBV reactivation requiring therapy with rituximab will be described using the Aalen-Johansen estimator, with death treated as a competing risk. An estimate and 95% CIs of the cumulative incidence of EBV-associated lymphoproliferative disorder/EBV reactivation will be provided at Day 180 post-initiation of T-Guard.

5.5.6 Incidence of Investigational Medical Product (IMP)-related Serious Adverse Events (SAEs)

SAEs that are deemed to be related to T-guard treatment will be summarized by type, frequency, and number of patients affected.

5.5.7 T-cell Subsets and NK-cells

The following cell populations will be measured by flow cytometry analysis: Inflammatory Monocytes & Dendritic Cells, Recent Thymic Emigrants, CD4+, CD8+ Naïve & Memory Cells, CD4+ T Regulatory Cells, NK Cells, γδ T Cells, and B cells. These cells will be obtained from blood samples drawn at Days 0, 2, 4, 6 (just prior to and 4 hours after each T-Guard infusion) and at Days 14, 28, 56, and 180. The level of each population will be summarized using descriptive statistics at each assessment time considered. Changes in levels from baseline will be evaluated using Wilcoxon signed rank tests.

5.5.8 GVHD-related Biomarkers

Using the biomarker risk model from (Major-Monfried, Renteria et al. 2018), the serum levels of REG3α and ST2, and the 3-IS urine levels at enrollment and Day 7, 14, and 28 post-initiation of T-Guard will be used to estimate NRM probabilities for each patient at Day 180 following each assessment time point. The proportions of patients with high risk (defined as an estimated NRM greater than 0.29) will be described at each assessment time.

Moreover, the proportions of patients experiencing a Day 28 CR will be described separately for patients with high risk vs. not using sample proportions and 95% Wilson score or Clopper-Pearson CIs, as appropriate.

5.5.9 Patient-reported Outcomes

Patient self-reported measures will be assessed using selected PROMIS subscales for GI symptoms, physical function and satisfaction with participation in social roles. These instruments will be scored according to the recommendations of the developers. Scores at baseline and at Day 28, 56, and 180 will be described using descriptive statistics. Wilcoxon signed rank tests will be used to evaluate changes from baseline to each post-enrollment time point.

APPENDIX A: HUMAN SUBJECTS

1. Subject Consent

Candidates for the study will be identified as described in Chapter 4 of the protocol. The Prinicipal Investigator or his/her designee at each transplant center will contact the candidates, provide the patient with information about the purpose of the study, and obtain consent. The BMT CTN will provide a template of the consent form to each center. Each center will customize the templates according to their local requirements and submit for review by the DCC for adequacy prior to submitting to the NMDP IRB of Record. Each center must provide evidence of IRB approval to the DCC.

2. Confidentiality

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

3. Participation of Children, Women and Minorities

Women, ethnic minorities, children at least 12 and less 18 years of age, and other populations will be included in this study. Children less than 12 years of age are not eligible for this study given lack of applicable safety and PK data with T-Guard. 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 high risk acute GVHD in these groups. Centers will be notified if their rates differ significantly from those expected and asked to develop appropriate recruitment reports.

APPENDIX B: LABORATORY PROCEDURES

Collection of Mandatory Research Samples for Protocol-Defined Correlative Studies Research samples will be collected for patients who consent to the BMT CTN 1802 study. Required research samples for study-specific exploratory endpoints include the collection of peripheral blood and urine samples as summarized in the table below. The following planned studies will be performed and remaining biospecimens made available to approved investigators for meritorious ancillary correlative laboratory studies with the potential to extend the findings of the current study portfolio.

- Analysis of serum cytokines before and after T-Guard infusions
- Evaluation of serum and urine biomarkers associated with acute GVHD
- PK of T-Guard - development of a population pharmacokinetic model
- Evaluation of potential humoral responses (ADA) to T-Guard infusions
- Evaluation of T-Guard treatment on targeted and non-target immune cell subsets and presence of alloreactive and CMV/EBV specific T-cells

Once the samples are collected at specified time points they will either be shipped on the day of collection directly to specified project laboratory for testing or to the BMT CTN Biorepository for processing/long-term aliquot storage. In some cases, the samples will be transferred internally to a laboratory for local processing and freezing and then periodically batch shipped to the BMT CTN Biorepository for long-term storage and distribution for planned and approved studies. The collection and shipment of these blood and urine samples will be tracked using the network's GlobalTrace sample management system. Detailed procedures regarding specimen collection schedules, sample handling/processing procedures and shipping instructions will be found in the BMT CTN 1802 Research Sample Information Guide.

Subjects	Sample Type	Sample Collection Time Points	Sample Collection and Sample Processing Summary	Shipping Specifications	Shipping and Testing Locations
Patients	**Peripheral Blood** (SST Clot Tube) **5 mL** **Humoral Response Serum Anti-Drug Antibodies**	**Pre-treatment initiation** Day -3 to Day 0 **Post-treatment initiation** Days 7, 14, 28, 90 and 180	Collect the blood sample and place 5 mL in SST Vacutainer tube containing no anticoagulant. Allow blood samples to clot upright for 30-60 minutes in a tube rack prior to centrifugation. Once centrifuged, the tube will be packaged for same-day shipping to Biorepository. Refer to the BMT CTN 1802 Research Specimen Information Guide for details related to sample centrifugation and shipping.	Centrifuged blood sample tube will be shipped at 2-8ºC on the day of collection, to the BMT CTN Biorepository by priority overnight FedEx® delivery.	BMT CTN Biorepository
Patients	**Peripheral Blood**[1] (SST Clot Tube) **5 mL** **Serum Pharmacokinetic Testing**	**Day 0** **(1st T-Guard infusion)** Prior to study drug infusion *and* 4, 5, 6, 8, and 24 hours after start of infusion **Days 2 & 4** **(2nd & 3rd infusions)** Prior to study drug infusion *and* 4, 6, and 24 hours after start of infusion **Day 6** **(4th infusion)** Prior to study drug infusion *and*	Collect the blood sample and place 5 mL in SST Vacutainer tube containing no anticoagulant. Allow blood samples to clot upright for 30-60 minutes in a tube rack prior to centrifugation, serum removal and serum aliquot storage at -80ºC. Refer to the BMT CTN 1802 Research Specimen Information Guide for details related to sample processing, aliquot labeling, storing and shipping.	Frozen serum sample aliquots will be periodically batched-shipped to the BMT CTN Biorepository by priority overnight FedEx® delivery.	BMT CTN Biorepository

		4, 6, 24, and 48 hours after start of infusion			

Required Research Sample Summary

Subjects	Sample Type	Sample Collection Time Points	Sample Collection and Sample Processing Summary	Shipping Specifications	Shipping and Testing Locations
Patients	**Peripheral Blood** (SST Clot Tube) **10 mL** **Serum** **Cytokines and GVHD Biomarkers**	**Pre-treatment initiation** Day -3 to Day 0 **Post-treatment initiation** Days 7, 14 and 28	Collect the blood sample and place 5 mL in SST Vacutainer tube containing no anticoagulant. Allow blood samples to clot upright for 30-60 minutes in a tube rack prior to centrifugation. Once centrifuged, the tube will be packaged for same-day shipping to Biorepository. Refer to the BMT CTN 1802 Research Specimen Information Guide for details related to sample centrifugation and shipping.	Centrifuged blood sample tube will be shipped at 2-8ºC on the day of collection, to the BMT CTN Biorepository by priority overnight FedEx® delivery.	BMT CTN Biorepository
Patients	**Peripheral Blood** (Sodium Heparin Vacutainer Tubes)	**Pre-treatment initiation**		Blood sample tubes will be shipped at 2- 8ºC on the day of collection, to the	BMT CTN Biorepository

	30 mL **Peripheral blood mononuclear cells (PBMC)** **CMV/EBV and Alloreactive T- Cells**	Day -3 to Day 0 **Post-treatment initiation** Days 28 and 180	Collect the blood sample and place 10 mL into each of three Vacutainer tubes containing sodium heparin anticoagulant. Gently mix sample by inversion 8-10 times to mix sample well with anticoagulant.	BMT CTN Biorepository by priority overnight FedEx® delivery.	

Subjects	Sample Type	Sample Collection Time Points	Sample Collection and Sample Processing Summary	Shipping Specifications	Shipping and Testing Locations
Patients	**Urine** (sterile transport tube) **10 mL** **GVHD Related Biomarkers**	**Pre-treatment initiation** Day -3 to Day 0 **Post-treatment initiation** Days 7, 14, and 28	Collect 10 mL urine and place in sterile transport tube for same-day shipping to Biorepository. Refer to the BMT CTN 1802 Research Specimen Information Guide for details related to sample centrifugation and shipping.	Urine sample tube will be shipped at 2- 8ºC on the day of collection, to the BMT CTN Biorepository by priority overnight FedEx® delivery.	BMT CTN Biorepository
Patients	**Peripheral Blood[2]** (Cyto-Chex BCT) **10 mL** **Immunophenotyping**	**Pre-treatment initiation** Day -3 to Day 0 **Day 0, 2, 4 and 6 1st through 4th T-Guard infusion** Prior to study drug infusion *and* 4 hours after infusion **Post-treatment initiation** Days 14, 28, 56, and 180	Collect the blood sample and place 5 mL into each of two Cyto-Chex tubes containing EDTA anticoagulant and cell fixative. Gently mix sample by inversion 8-10 times to mix sample well with anticoagulant and cell fixative reagent.	Cyto-Chex blood tubes will be shipped at 2-8ºC on the day of collection to the RPCI project laboratory by priority overnight FedEx® delivery.	Project Laboratory (RPCI)

[1]Pharmcokinetic blood samples directly pre-infusion, and at 4, 5, 6, 8, and 24 hours after start of 1st infusion, pre-infusion, and at 4, 6, and 24 hours after the 2nd and 3rd infusion, and pre-infusion, and at 4, 6, 24 and 48 hours after the 4th T-Guard infusion. Pre-infusion samples should be drawn prior to T-Guard administration. A window of +/- 15 minutes for samples drawn at 4,5, or 6 hours after the start of infusion, and a window of +/-30 minutes for the sample drawn 8 hours after the start of infusion is allowed. A window of +/- 2 hours for samples drawn 24 or 48 hours after the start of infusion is also allowed. Every effort should be made to collect PK samples at all timepoints, however, the treatment schedule should not be altered to accommodate PK sampling. For example, if T-Guard administration is scheduled for a weekend and PK sampling is not possible then administration should occur on schedule. In the event weekend PK sample collection is missed, collection of PK samples should be resumed at the next T- Guard infusion.

[2] Peripheral blood for immunophenotyping should be collected pre-infusion and at 4 hours after the start of each infusion. Pre- infusion samples should be drawn prior to T-Guard administration and a window of +/- 15 minutes is allowed for the sample drawn 4 hours after the start of infusion.

APPENDIX C: STAGING AND GRADING OF ACUTE GVHD (HARRIS, YOUNG ET AL. 2016)

Table 1
GVHD Target Organ Staging

Stage	Skin (Active Erythema Only)	Liver (Bilirubin)	Upper GI	Lower GI (stool output/day)
0	No active (erythematous) GVHD rash	<2 mg/dL	No or intermittent nausea, vomiting, or anorexia	Adult: <500 mL/day or <3 episodes/day Child: <10 mL/kg/day or <4 episodes/day
1	Maculopapular rash <25% BSA	2-3 mg/dL	Persistent nausea, vomiting or anorexia	Adult: 500-999 mL/day or 3-4 episodes/day Child: 10-19.9 mL/kg/day or 4-6 episodes/day
2	Maculopapular rash 25-50% BSA	3.1-6 mg/dL		Adult: 1000-1500 mL/day or 5-7 episodes/day Child: 20-30 mL/kg/day or 7-10 episodes/day
3	Maculopapular rash >50% BSA	6.1-15 mg/dL		Adult: >1500 mL/day or >7 episodes/day Child: >30 mL/kg/day or >10 episodes/day
4	Generalized erythroderma (>50% BSA) *plus* bullous formation and desquamation >5% BSA	>15 mg/dL		Severe abdominal pain with or without ileus or grossly bloody stool (regardless of stool volume).

Overall clinical grade (based on most severe target organ involvement):
Grade 0: No stage 1-4 of any organ.
Grade I: Stage 1-2 skin without liver, upper GI, or lower GI involvement.
Grade II: Stage 3 rash and/or stage 1 liver and/or stage 1 upper GI and/or stage 1 lower GI.
Grade III: Stage 2-3 liver and/or stage 2-3 lower GI, with stage 0-3 skin and/or stage 0-1 upper GI.
Grade IV: Stage 4 skin, liver, or lower GI involvement, with stage 0-1 upper GI.

APPENDIX D: GRADING OF CHRONIC GVHD (NIH CRITERIA, (JAGASIA, GREINIX ET AL. 2015)

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
PERFORMANCE SCORE: ☐ **KPS ECOG LPS**	☐ Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	☐ Symptomatic, ambulatory, capabl of self-care, >50% of waking hours ou of bed (ECOG 2, KPS or LPS 60-70%)	☐ Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
SKIN† **SCORE % BSA** ☐ *GVHD features to be scored by BSA:* **Check all that apply:** ☐ Maculopapular rash/erythema ☐ Lichen planus-like features ☐ Sclerotic features ☐ Papulosquamous lesions or ichthyosis ☐ Keratosis pilaris-like GVHD	☐ No BSA involved	☐ 1-18% BSA	☐ 19-50% BSA	☐ >50% BSA
SKIN FEATURES SCORE:	☐ No sclerotic features		☐ Superficial sclerotic features "not hidebound" (able to pinch)	**Check all that apply:** ☐ Deep sclerotic features ☐ "Hidebound" (unable to pinch) ☐ Impaired mobility ☐ Ulceration
Other skin GVHD features (NOT scored by BSA) **Check all that apply:** ☐ Hyperpigmentation ☐ Hypopigmentation ☐ Poikiloderma ☐ Severe or generalized pruritus ☐ Hair involvement ☐ Nail involvement ☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				
MOUTH *Lichen planus-like features present:* ☐ **Yes** ☐ **No**	☐ No symptoms	☐ Mild symptoms **with** disease signs but not limiting oral intake significantly	☐ Moderate symptoms with disease signs **with** partial limitation of oral intake	☐ Severe symptoms with disease signs on examination **with** major limitation of oral intake
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				

Organ scoring of chronic GVHD. ECOG indicates Eastern Cooperative Oncology Group; KPS, Karnofsky Performance Status; LPS, Lansky Performance Status; BSA, body surface area; ADL, activities of daily living; LFTs, liver function tests; AP, alkaline phosphatase; ALT, alanine aminotransferase; ULN, normal upper limit. *Weight loss within 90 days. Skin scoring should use both percentage of BSA involved by disease signs and the cutaneous features scales. When a discrepancy exists between the percentage of total body surface (BSA) score and the skin feature score, OR if superficial sclerotic features are present (Score 2), but there is impaired mobility or ulceration (Score 3), the higher level should be used for the final skin scoring. To be completed by specialist or trained medical providers. **Lung scoring should be performed using both the symptoms and FEV1 scores whenever possible. FEV1 should be used in the final lung scoring where there is discrepancy between symptoms and FEV1 scores.

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
EYES *Keratoconjunctivitis sicca (KCS) confirmed by ophthalmologist:* ☐ **Yes** ☐ **No** ☐ **Not examined**	☐ No symptoms	☐ Mild dry eye symptoms not affecting ADL (requirement of lubricant eye drops ≤ 3 x per day)	☐ Moderate dry eye symptoms partially affecting ADL (requiring lubricant eye drops > 3 x per day or punctal plugs), **WITHOUT** new vision impairment due to KCS	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision due to KCS
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				
GI Tract ***Check all that apply:*** ☐ Esophageal web/ proximal stricture or ring ☐ Dysphagia ☐ Anorexia ☐ Nausea ☐ Vomiting ☐ Diarrhea ☐ Weight loss ≥5%* ☐ Failure to thrive	☐ No symptoms	☐ Symptoms without significant weight loss* (<5%)	☐ Symptoms associated with mild to moderate weight loss* (5-15%) **OR** moderate diarrhea without significant interference with daily living	☐ Symptoms associated with significant weight loss* >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation **OR** severe diarrhea with significant interference with daily living
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):* _____				
LIVER	☐ Normal total bilirubin and ALT or AP < 3 x ULN	☐ Normal total bilirubin with ALT ≥3 to 5 x ULN or AP ≥ 3 x ULN	☐ Elevated total bilirubin but ≤3 mg/dL or ALT > 5 ULN	☐ Elevated total bilirubin > 3 mg/dL
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				

LUNGS** **Symptom score**:	☐ No symptoms	☐ Mild symptoms (shortness of breath after climbing one flight of steps)	☐ Moderate symptoms (shortness of breath after walking on flat ground)	☐ Severe symptoms (shortness of breath at rest; requiring 0_2)
Lung score: **% FEV1** ☐	☐ FEV1≥80%	☐ FEV1 60-79%	☐ FEV1 40-59%	☐ FEV1 ≤39%

Pulmonary function tests
☐ Not performed
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):* _____

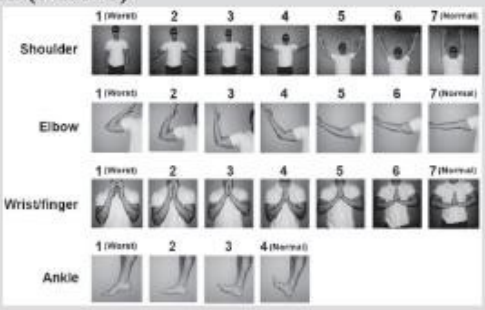
	SCORE 0	SCORE 1	SCORE 2	SCORE 3
JOINTS AND FASCIA P-ROM score *(see below)* Shoulder (1-7):___ Elbow (1-7): ____ Wrist/finger (1-7):___ Ankle (1-4):___	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	☐ Contractures **WITH** significant decrease of ROM ***AND*** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
GENITAL TRACT (*See Supplemental figure*‡) ☐ Not examined *Currently sexually active* ☐ Yes ☐ No	☐ No signs	☐ Mild signs‡ and females with or without discomfort on exam	☐ Moderate signs‡ and may have symptoms with discomfort on exam	☐ Severe signs‡ with or without symptoms
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				

Other indicators, clinical features or complications related to chronic GVHD (check all that apply and assign a score to severity (0-3) based on functional impact where applicable none – 0,mild -1, moderate -2, severe – 3)

☐ Ascites (serositis)___	☐ Myasthenia Gravis___	
☐ Pericardial Effusion___	☐ Peripheral Neuropathy___	☐ Eosinophilia > 500/µl___
☐ Pleural Effusion(s)___	☐ Polymyositis___	☐ Platelets <100,000/µl ___
☐ Nephrotic syndrome___	☐ Weight loss>5%* without GI symptoms___	☐ Others (specify):_____

Overall GVHD Severity *(Opinion of the evaluator)*	☐ **No GVHD**	☐ **Mild**	☐ **Moderate**	☐ **Severe**

Photographic Range of Motion (P-ROM)



APPENDIX E: PATIENT REPORTED OUTCOMES

In general, would you say your health is….

1. Poor
2. Fair
3. Good
4. Very good
5. Excellent

In the past 7 days, how often did you have nausea—that is, a feeling like you could vomit?

1. Never (skip next question)
2. Rarely
3. Sometimes
4. Often
5. Always

In the past 7 days, how often did you know that you would have nausea before it happened?

1. Never
2. Rarely
3. Sometimes
4. Often
5. Always

In the past 7 days, how often did you have a poor appetite?

1. Never
2. Rarely
3. Sometimes
4. Often
5. Always

In the past 7 days, how often did you throw up or vomit?

1. Never
2. One day
3. 2-6 days
4. Once a day
5. More than once a day

In the past 7 days, how often did you have belly pain?

1. Never
2. One day
3. 2-6 days
4. Once a day
5. More than once a day

In the past 7 days, how many days did you have loose or watery stools?

1. No days
2. One day
3. Two days
4. 3-5 days
5. 6-7 days

In the past 7 days, how often did you feel like you needed to empty your bowels right away or else you would have an accident?

1. Never
2. One time during the past 7 days
3. 2-6 days during the past 7 days
4. Often once a day
5. More than once a day

In the past 7 days, how often did you have bowel incontinence—that is, have an accident because you could not make it to the bathroom in time?

1. No days
2. One day
3. 2-3 days
4. 4-5days
5. 6-7 days

Are you able to dress yourself, including tying shoelaces and buttoning your clothes?

1. Without any difficulty
2. With a little difficulty
3. With some difficulty
4. With much difficulty
5. Unable to do

Are you able to get out of bed into a chair?

1. Without any difficulty
2. With a little difficulty
3. With some difficulty
4. With much difficulty
5. Unable to do

Are you able to go for a walk of at least 15 minutes?

1. Without any difficulty
2. With a little difficulty
3. With some difficulty
4. With much difficulty
5. Unable to do

Are you able to go up and down stairs at a normal pace?

1. Without any difficulty
2. With a little difficulty
3. With some difficulty
4. With much difficulty
5. Unable to do

In the past 7 days, I feel fatigued

1. Not at all
2. A little bit
3. Somewhat
4. Quite a bit
5. Very much

In the past 7 days, I have trouble starting things because I am tired

1. Not at all
2. A little bit
3. Somewhat
4. Quite a bit
5. Very much

In the past 7 days, how run-down did you feel on average?

1. Not at all
2. A little bit
3. Somewhat
4. Quite a bit
5. Very much

In the past 7 days, how fatigued were you on average?

1. Not at all
2. A little bit
3. Somewhat
4. Quite a bit
5. Very much

In the past 7 days, how much did pain interfere with your day to day activities?

1. Not at all
2. A little bit
3. Somewhat
4. Quite a bit
5. Very much

In the past 7 days, how much did pain interfere with your enjoyment of life?

1. Not at all
2. A little bit
3. Somewhat
4. Quite a bit
5. Very much

In the past 7 days, how would you rate your pain on average?

0. No pain 0 1.
1
2. 2
3. 3
4. 4
5. 5
6. 6
7. 7
8. 8
9. 9
10. Worst imaginable pain, 10

In the past 7 days, how bothered were you by sores on your skin?

1. Not at all
2. Slightly
3. Moderately
4. Quite a bit
5. Extremely

In the past 7 days, how intense was your itch in general?

1. Had no itch
2. Mild
3. Moderate
4. Severe
5. Very severe

In the past 7 days, I felt worthless

1. Never
2. Rarely
3. Sometimes
4. Often
5. Always

In the past 7 days, I felt helpless

1. Never
2. Rarely
3. Sometimes
4. Often
5. Always

In the past 7 days, I felt depressed

1. Never
2. Rarely
3. Sometimes
4. Often
5. Always

In the past 7 days, I felt hopeless

1. Never
2. Rarely
3. Sometimes
4. Often
5. Always

In the past 7 days, my sleep quality was…

1. Very poor
2. Poor
3. Fair
4. Good
5. Very good

In the past 7 days, my sleep was refreshing

1. Not at all
2. A little bit
3. Somewhat
4. Quite a bit
5. Very much

In the past 7 days, I had a problem with my sleep

1. Not at all
2. A little bit
3. Somewhat
4. Quite a bit
5. Very much

In the past 7 days, I had difficulty falling asleep

1. Not at all
2. A little bit
3. Somewhat
4. Quite a bit
5. Very much

APPENDIX F: LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AAT	Alpha-1 Antitrypsin
ADA	Anti-Drug-Antibody
AE	Adverse Event
Allo-HSCT	Allogeneic Hematopoietic Stem Cell Transplantation
ATG	Anti-Thymocyte Globulin
AUC	Areas Under the Time-Concentration Curves
B-cells	B Lymphocytes
BSA	Body Surface Area
C_{max}	Peak Concentration
CBC	Complete Blood Count
CD3/TCR	CD3 T-Cell Receptor
CI	Confidence Interval
CIBMTR	Center for International Blood and Marrow Transplant Research
CK	Creatine Kinase
CLL	Chronic Lymphocytic Leukemia
CLS	Capillary Leak Syndrome
CMV	Cytomegalovirus
CR	Complete Response, the disappearance of symptoms in all organ systems
CRS	Cytokine Release Syndrome
eCRFs	electronic Case Report Forms
CTCAE	Common Terminology Criteria for Adverse Events
DoCR	Duration of Complete Response
DRI	Disease Risk Index
DSMB	Data and Safety Monitoring Board
EBV	Epstein-Barr Virus
EDC	Electronic Data Capture
ERC	Endpoint Review Committee
GCP	Good Clinical Practice
GI	Gastro-intestinal
GVHD	Graft-Versus-Host Disease
aGVHD	(acute) Graft-Versus-Host Disease
cGVHD	(chronic) Graft-Versus-Host Disease
SR-aGVHD	Steroid-refractory (acute) Graft-Versus-Host Disease
FDG	Fluoro-deoxyglucose
HCT	Hematopoietic Cell Transplantation
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IFN	Interferon
Ig	Immunoglobulin

List of Abbreviations and Definitions of Terms

IL	Interleukin
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
JAK	Janus Kinase
mAb	Monoclonal Antibody
NIH	National Institutes of Health (USA)
NK cells	Natural Killer Cells
NR	Non-Responder; No Response, Progression/Relapse of aGVHD or death by the end of Day 28
NRM	Non-Relapse Mortality
ORR	Overall Response Rate
OS	Overall Survival
PBMC	Peripheral Blood Mononuclear Cells
PD	Progressive Disease, progression in 1 or more organ-systems resulting in a worsening of overall at least one Grade, without improvement in any other organs
PET	Positron Emission Tomography
PHA	Phytohemagglutinin
PK	Pharmacokinetics
PP	Per Protocol
PR	Partial Response, the improvement of 1 or more organs, with no worsening in other organs
Progression/Malignancy Relapse	The time from the date of the start of treatment to the date of hematologic malignancy relapse/progression.
`α	Regenerating Family Member 3 Alpha
RFS	Relapse Free Survival
RTA	Ricin Toxin A-chain
SAE	Serious Adverse Event
SoC	Standard of Care
SPRT	Sequential Probability Ratio Test
SPV-T3a	Anti-CD3, IgG2b
SR	Steroid refractory
ST2	Interleukin 1 receptor-like 1
TEAE	Treatment Emergent Adverse Event
$t_{1/2}$	Terminal-phase Elimination Half-life
T cells	T Lymphocytes
TCR	T-cell Receptor
WT1	Anti-CD7, IgG2a
3-IS	3-Indoxyl Sulfate

APPENDIX G: REFERENCES

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