BLOOD AND MARROW TRANSPLANT CLINICAL TRIALS NETWORK

A Phase 3, Randomized, Open-Label, Multicenter Study, to Compare T-Guard to Ruxolitinib for the Treatment of Patients with Grade III or IV Steroid-Refractory Acute Graft-Versus-Host Disease (SR-aGVHD)

(Posted on ClinicalTrials.gov as NCT04934670)
BMT CTN PROTOCOL 2002
Version 1.0

Study Chairpersons
John Levine, MD[1], Gabrielle Meyers, MD[2], and Gérard Socié, MD, PhD[3]

Protocol Officer
Mehdi Hamadani, MD[4]

Protocol Team

Haris Ali, MD[5]
Hannah Choe, MD[6]
Andrew Harris, MD[7]
Ernst Holler, MD[8]
Eric van Hooren[9]
Denise King, MS[10]
Willem Klaassen[9]
Eric Leifer, PhD[11]
Michael Martens, PhD[4]
Ypke van Oosterhout, PhD[9]
Lia Perez, MD[12]
Iskra Pusic, MD[13]
Jenn Romeril, RN[10]
Matthias Stelljes, MD[14]
Walter Van der Velden, MD, PhD[15]
Heather Wittsack, MPH[10]

Sponsored by the National Institutes of Health
National Heart, Lung, and Blood Institute
National Cancer Institute
Xenikos BV
IND # 122098
EudraCT # 2021-000343-53

[1]Mount Sinai Medical Center
[2]Oregon Health and Science University
[3]AP-HP Hospital Saint Louis, Paris
[4]Medical College of Wisconsin
[5]City of Hope National Medical
[6]Ohio State University
[7]University of Utah
[8]Universitatsklinikum Regensburg
[9]Xenikos BV
[10]The Emmes Company
[11]National Heart, Lung, and Blood Institute
[12]H. Lee Moffitt Cancer Center
[13]Washington University School of Medicine
[14]Universitatsklinikum Munster
[15]Radboud University

John Levine
John Levine I am approving this document. 06/29/2021 02:23 PM EDT
Approval Signature (Protocol Chair)

Gabrielle Meyers
Gabrielle Meyers I am approving this document. 06/29/2021 07:37 PM EDT
Approval Signature (Protocol Chair)

Gerard Socie
Gerard Socie I am approving this document. 06/30/2021 11:05 AM EDT
Approval Signature (Protocol Chair)

Mehdi Hamadani
Mehdi Hamadani I am approving this document. 07/01/2021 02:34 PM EDT
Approval Signature (Protocol Officer)

Ypke van Oosterhout
Ypke van Oosterhout I am approving this document. 06/30/2021 10:13 AM EDT
Approval Signature (Xenikos)

PROTOCOL SYNOPSIS – BMT CTN 2002 PROTOCOL

A Phase 3, Randomized, Open-Label, Multicenter Study, to Compare T-Guard to Ruxolitinib for the Treatment of Patients with Grade III or IV Steroid-Refractory Acute Graft-Versus-Host Disease (SR-aGVHD)

Co-Chairs:

John Levine, MD,
Gabrielle Meyers, MD,
Gérard Socié, MD, PhD

Study Design:

The study is an open-label, randomized, Phase 3, multicenter trial, which has been designed to compare the efficacy and safety of T-Guard to ruxolitinib in patients with Grade III or IV Steroid-Refractory acute Graft-Versus-Host Disease (SR-aGVHD).

The primary analysis will include all participants that are randomized.

Primary Objective:

To assess the rate of complete response (CR) in Grades III and IV SR-aGVHD participants on Day 28 post-randomization.

Secondary Objectives:

Secondary objectives are the following:

1. Estimate the overall survival (OS) at Days 60, 90, and 180.
2. Evaluate the duration of complete response (DoCR).
3. Estimate the time to complete response (CR) from randomization.
4. Estimate the overall response rate (CR or partial response (PR)) at Days 14, 28, and 56.
5. Describe proportions of CR, PR, mixed response (MR), no response (NR), and progression of aGVHD at Days 6, 14, 28, and 56.
6. Estimate the cumulative incidence of non-relapse mortality (NRM) at Days 90 and 180.
7. Estimate relapse-free survival at Day 180.
8. Estimate GVHD-free survival at Days 90 and 180.
9. Estimate the cumulative incidence of chronic GVHD (cGVHD) at Day 180.
10. Estimate the cumulative incidence of underlying disease relapse/progression at Day 180.
11. Describe the incidence of infections.
12. Describe the incidence of toxicities.
13. Assess the pharmacokinetics (PK) of T-Guard.
14. Assess the immunogenicity of T-Guard.

Exploratory Objectives:

Exploratory objectives are the following:

1. Describe proportion of participants free of systemic steroids by Day 180 post-randomization.
2. Estimate the incidence of Cytomegalovirus (CMV) reactivation requiring therapy by Day 180 post-randomization.
3. Estimate the incidence of Epstein-Barr Virus (EBV)-associate lymphoproliferative disorder or EBV reactivation requiring therapy with rituximab by Day 180 post-randomization.
4. Evaluate the evolution and characteristics of specific cell populations at randomization and Days 0, 14, 56, and 180.
5. Evaluate aGVHD biomarkers at baseline and at Days 6, 14, and 28 post-randomization.
6. Describe changes in patient-reported outcomes (PROs) from baseline to Days 28, 90, and 180 post-randomization.
7. Estimate incidence of TMA at Days 6, 14, 21 and 28 post-randomization.
8. Describe EASIX score at screening.

Correlatives:

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

Eligibility Criteria:

Inclusion Criteria:

1. Patients must be at least 18.0 years of age at the time of consent.
2. Patient has undergone first allo-HSCT from any donor source or graft source.
3. Patients diagnosed with Grade III or IV SR-aGVHD after allo-HSCT. SR includes aGVHD initially treated at a lower steroid dose, but must meet one of the following criteria:
    - progressed or new organ involvement after 3 days of treatment with methylprednisolone (or equivalent) of greater than or equal to 2 mg/kg/day,
    - no improvement after 7 days of primary treatment with methylprednisolone (or equivalent) of greater than or equal to 2mg/kg/day
    - patients with visceral (GI and/or liver) plus skin aGVHD at methylprednisolone (or equivalent) initiation with improvement in skin GVHD without any improvement in visceral GVHD after 7 days of primary treatment with methylprednisolone (or equivalent) of greater than or equal to 2mg/kg/day
    - Patients who have skin GVHD alone and develop visceral aGVHD during treatment with methylprednisolone (or equivalent) of greater than or equal to 1mg/kg/day and do not improve after 3 days

of greater than or equal to 2mg/kg/day

4. Patients must 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.
5. 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:

1. Patients who have a creatinine greater than or equal to 2mg/dL or estimated creatinine clearance less than 40 mL/min or those requiring hemodialysis.
2. Patients who have been diagnosed with active Thrombotic Microangiopathy (TMA), defined as meeting **all** the following criteria:
   - greater than 4% schistocytes in blood (or equivalent if semiquantitative scale is used e.g., 3+ or 4+ schistocytes on peripheral blood smear),
   - de novo, prolonged or progressive thrombocytopenia (platelet count less than 50 x 10^9/L or 50% or greater reduction from previous counts),
   - sudden and persistent increase in lactate dehydrogenase concentration greater than 2x ULN,
   - decrease in hemoglobin concentration or increased transfusion requirement attributed to Coombs-negative hemolysis, AND
   - decrease in serum haptoglobin
3. Patients who have previously received treatment with eculizumab.
4. Patients who have previously received checkpoint inhibitors (either before or after allo-HCT).
5. Patients who have been diagnosed with overlap syndrome, that is, with any concurrent features of cGVHD.
6. Patients requiring mechanical ventilation or vasopressor support.
7. Patients who have received any systemic treatment, besides steroids, as upfront treatment of aGVHD or as treatment for SR-aGVHD. Reinstitution of previously used GVHD prophylaxis agents (e.g., tacrolimus, cyclosporin, MTX, MMF) or substitutes in cases with previously documented intolerance will be permitted. Previous treatment with a JAK inhibitor as part of GVHD prophylaxis or treatment is not allowed.
8. Patients who have severe hypoalbuminemia, with an albumin of less than or equal to 1 g/dl.

9. Patients who have a creatine kinase (CK) level of greater than 5 times the upper limit of normal.
10. 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. Persisting fever without other signs or symptoms will not be interpreted as progressing infection. Progression of infection is defined as:
    - hemodynamic instability attributable to sepsis OR
    - new symptoms attributable to infection OR
    - worsening physical signs attributable to infection OR
    - worsening radiographic findings attributable to infection
11. Patients with evidence of relapsed, progressing, or persistent malignancy, or who have been treated for relapse after transplant, or who may require rapid immune suppression withdrawal as pre-emergent treatment of early malignancy relapse.
12. Patients with evidence of minimal residual disease requiring withdrawal of systemic immune suppression.
13. Patients with unresolved serious toxicity or complications (other than acute GVHD) due to previous transplant.
14. History of sinusoidal obstruction syndrome (SOS)/veno-occlusive disease (VOD).
15. Patients with known hypersensitivity to any of the components murine monoclonal antibodies (mAb) or recombinant Ricin Toxin A-chain (RTA).
16. Patients who have had treatment with any other investigational agent, device, or procedure within 21 days (or 5 half-lives, whichever is greater) prior to enrollment.
17. Patients who have received more than one allo-HSCT.
18. Patients with known human immunodeficiency virus infection.
19. Patients who have a BMI greater than or equal to 35 kg/m^2.
20. Patients who are taking sirolimus must have it discontinued prior to starting study treatment.
21. 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 treatment dose.
22. 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 treatment dose.
23. 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.

24. 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.

Interim Analysis: This trial will include one interim analysis for futility after 23 participants on the T-Guard arm (~46 participants total) become evaluable for the primary endpoint. One interim analysis for efficacy will be performed once 150 participants on combined arms have reached Day 28 and 100 participants on the combined arms have reached Day 180.

Treatment Description: Participants will be randomized to receive either T-Guard or ruxolitinib. Participants on the T-Guard arm 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). Participants on the ruxolitinib arm will receive 10mg orally of ruxolitinib twice daily for a planned minimal period of 56 days.

Accrual Objective: The target accrual is 246 participants randomized 1:1 between the treatment arms from approximately 75 transplant centers in the US and Europe.

Accrual Period: Approximately 34 months is expected for accrual.

Study Duration: Participants will be followed for 180 days after randomization for a total study duration of approximately 40 months.

Safety Monitoring: A safety run-in phase of 12 T-Guard participants (~24 on the combined treatment arms) will begin the trial, with two comprehensive safety reviews conducted by the DSMB after 6 and 12 T-Guard treated participants reach Day 60. The rates of early overall mortality post randomization will be monitored using sequential probability ratio tests (SPRT) for binary data. Two SPRTs will be implemented: one contrasting a Day 30 mortality rate of 15% vs. a 30% rate in the T-Guard arm specifically, and another evaluating whether excessive Day 60 mortality risk is present in the T-Guard arm compared to ruxolitinib.

Outline of Treatment Plan

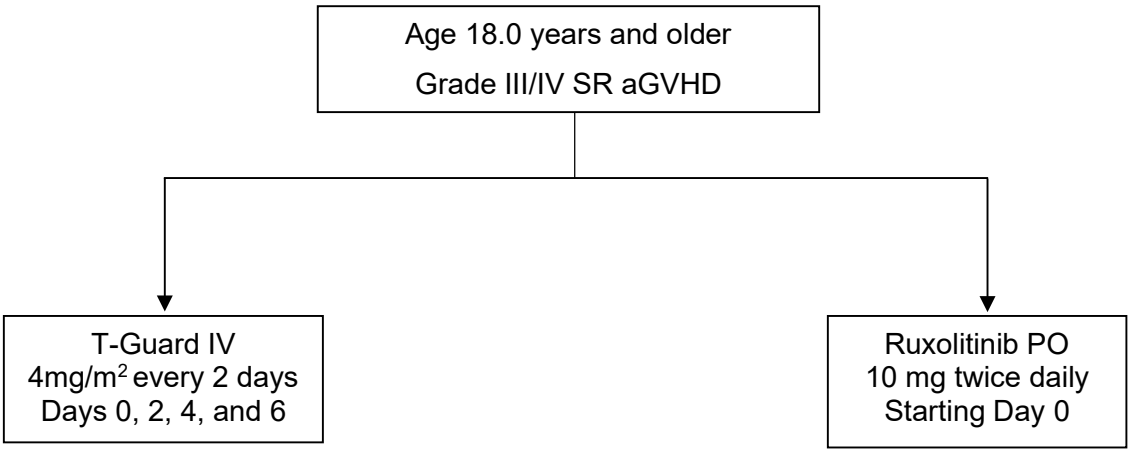


TABLE OF CONTENTS

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 two main categories of GVHD, each with two 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 pathology, 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, Hill 2018, Zeiser and Blazar 2017). 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 the absence of skin rash, diarrhea and hyperbilirubinemia (Hings, Severson et al. 1994, MacMillan, Weisdorf et al. 2002, Deeg 2007). With regards to second-line treatment, data from the prospective ruxolitinib treatment trials (REACH1 and REACH2), as well as from a prospective natural history trial (MAGIC), found the expected CR rate at day 28 after second-line therapy therapy is in the range of 25%-30%. The likelihood of GVHD treatment response decreases with increasing severity of the disease. (Martin, Schoch et al. 1990, Weisdorf, Haake et al. 1990). The response, more particular: Complete Response, to 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 (Pidala, Hamadani et al 2020). 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 SR-aGVHD, and increases in steroid dosing do not improve survival, (Bacigalupo, van Lint et al. 1983), thus another therapeutic agent/intervention is added to the treatment regimen.

Though substantial progress has been made in the field over 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 who 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), attributed to poor response rates with second-line treatment (Deeg 2007, Pidala, Kim et al. 2010, Castilla-Llorente, Martin et al. 2014), and infectious complications due to profound immunosuppression.

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, be effective upfront to avoid severe and irreversible tissue damage, and provide

complete and durable responses without impacting graft versus tumor effects and/or infection risk.

Ruxolitinib

The dual Janus Kinases (JAKs) JAK1 and JAK2 inhibitor ruxolitinib has shown efficacy and tolerability in the upfront treatment of aGVHD and SR-aGVHD. The pivotal phase 2 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. The REACH 2 randomized trial comparing Ruxolitinib versus best available treatment (BAT) has been published (Zeiser, Bubnoff et al. 2020.). This multicenter open-label phase 3 trial compared the efficacy and safety of oral ruxolitinib (10 mg twice daily) with the investigator's choice of therapy from a list of nine commonly used options (control) in patients 12 years of age or older who had SR-aGVHD after allogeneic stem-cell transplantation. The primary end point was overall response (complete response or partial response) at day 28. The key secondary end point was durable overall response at day 56.

A total of 309 patients underwent randomization; 154 patients were assigned to the ruxolitinib group and 155 to the control group. Ruxolitinib was superior to BAT by multiple measures of efficacy including overall response at day 28 (62% vs. 39%; P<0.001), maintenance of response to day 56 (40% vs. 22%; P<0.001), estimated cumulative incidence of loss of response at 6 months (10% vs. 39%), and median failure-free survival (5.0 vs. 1.0 month; hazard ratio 0.46; 95% CI, 0.35 to 0.60). The median overall survival (OS) was significantly longer for the ruxolitinib group (11.1 vs. 6.5 months; hazard ratio for death, 0.83; 95% CI, 0.60 to 1.15). The most common adverse events up to day 28 were thrombocytopenia (ruxolitinib 33%; controls 18%, anemia (ruxolitinib 30%; controls 28%), and cytomegalovirus (CMV) infection (ruxolitinib 26%; controls 21%).

Results of ruxolitinib in SR-aGVHD have been recently reviewed (Socie & Zeiser Blood advances 2020) and new criteria have been proposed for ruxolitinib-refractory aGVHD after this pivotal trial (Mohty, M., Holler, E., 2020). Thus, ruxolitinib is currently the sole treatment that has been reported to improve response rate over BAT in the setting of a randomized phase 3 trial.

Monoclonal Antibodies (mAbs)

Other treatments for SR-aGVHD have included mAbs, such as ABX-CBL or an anti-interleukin receptor antibody (Leukotac) but these have failed to improve outcomes [reviewed in; Martin et al; Biol Blood Marrow Transplant 2012;18: 1150-1163] (Macmillan; Blood 2007; 109: 2657-2662. Socie; Blood 2017; 129: 643-649).

SUMMARY

Treatment failure and treatment-related toxicities remain major problems for patients with aGVHD despite numerous prior studies and the recent approval of ruxolitinib. The need for better treatment is especially important for patients at the highest risk for treatment failure, those with grade III/IV SR-aGVHD.

In this proposed study, a new immunotoxin will be investigated in patients with Grade III/IV SR-aGVHD and compared to ruxolitinib. This agent is attractive to study based on immunosuppressive profile and response rates reported on 20 patients in a Phase 1/2 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 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 due to the lack of ongoing exposure to immunosuppressive agents.

1.3 INVESTIGATIONAL MEDICINAL PRODUCTS

In this study T-Guard will be compared to ruxolitinib. Both products will be supplied by the sponsor including protocol-specific dosing instructions (see sections 2.5.1.4 and 2.5.2.3).

1.3.1 T-Guard

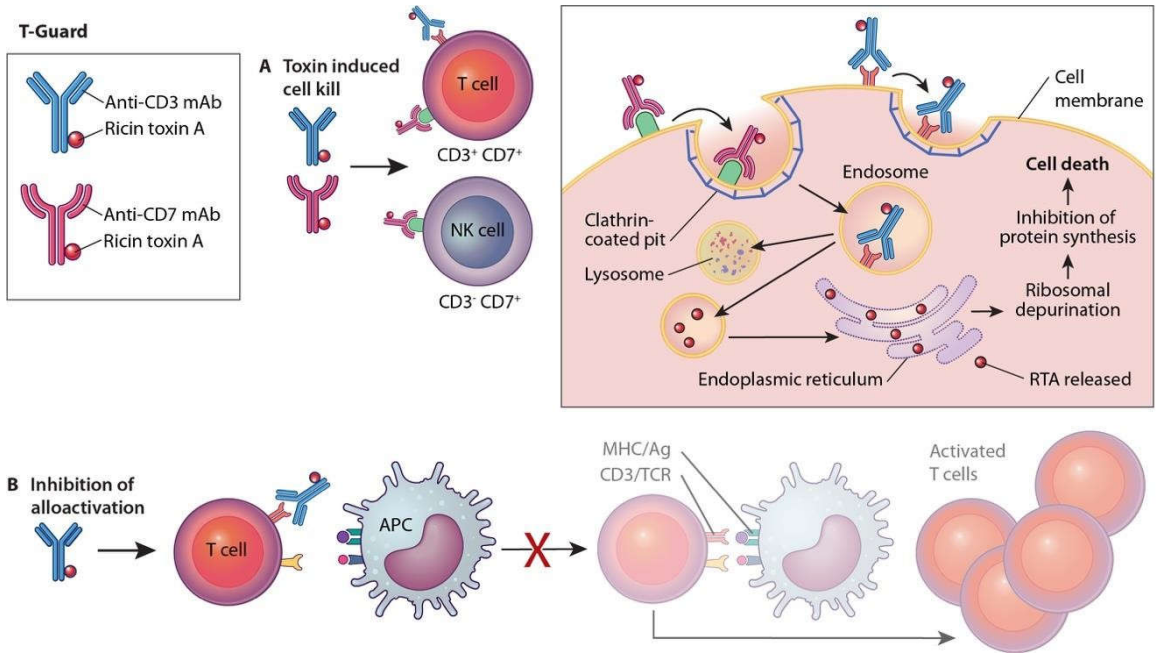
T-Guard is an immunotoxin-combination, consisting of two equal amounts (w/w) of the murine mAbs SPV-T3a (anti-CD3, IgG2b) and WT1 (anti-CD7, IgG2a), each conjugated to the Ricin Toxic A (RTA) chain: SPV-T3a-RTA and WT1-RTA. This particular immunotoxin-combination harbors multiple mechanisms of action (see 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 section 1.3.1.1). 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 to 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 section 1.3.1.1). 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



1.3.1.1 Pre-Clinical Data

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

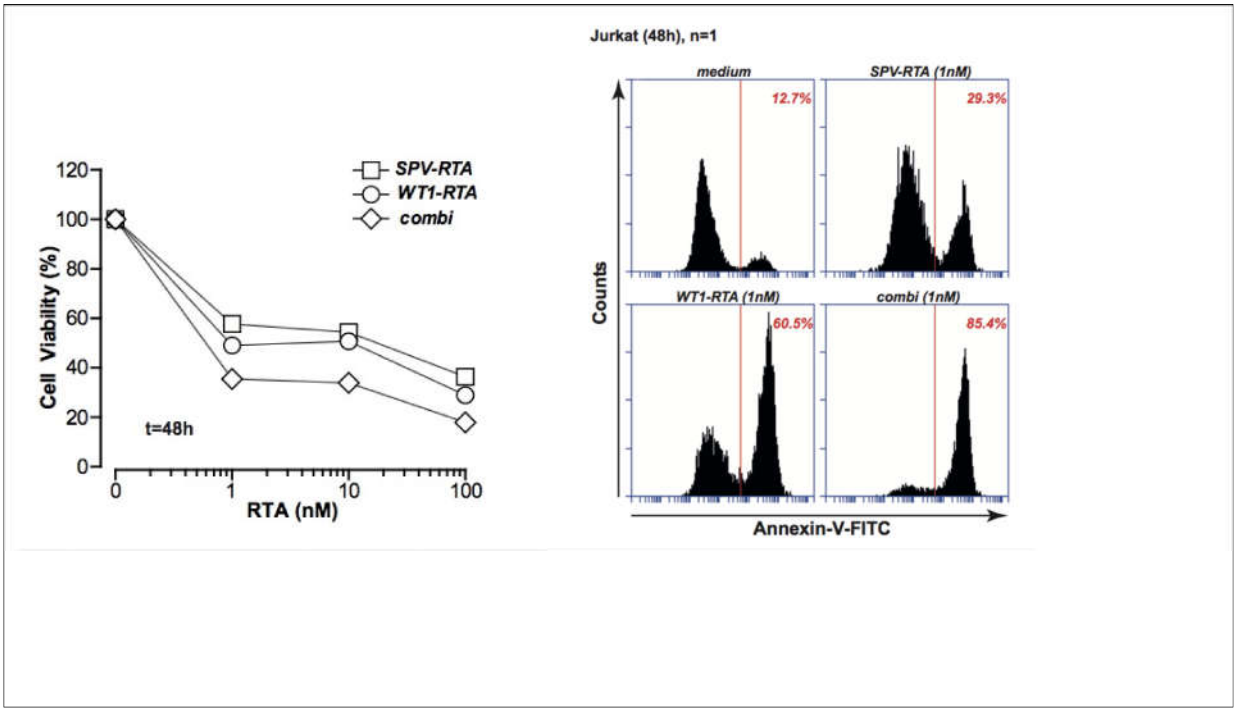
1.3.1.1.1 Synergistic Apoptosis of a Combination of Anti-T-Cell Immunotoxins

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 percent cell viability of Jurkat cells treated with either SPV-T3a-RTA and WT1- RTA or in combination (half a dose each).

1.3.1.1.2 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).

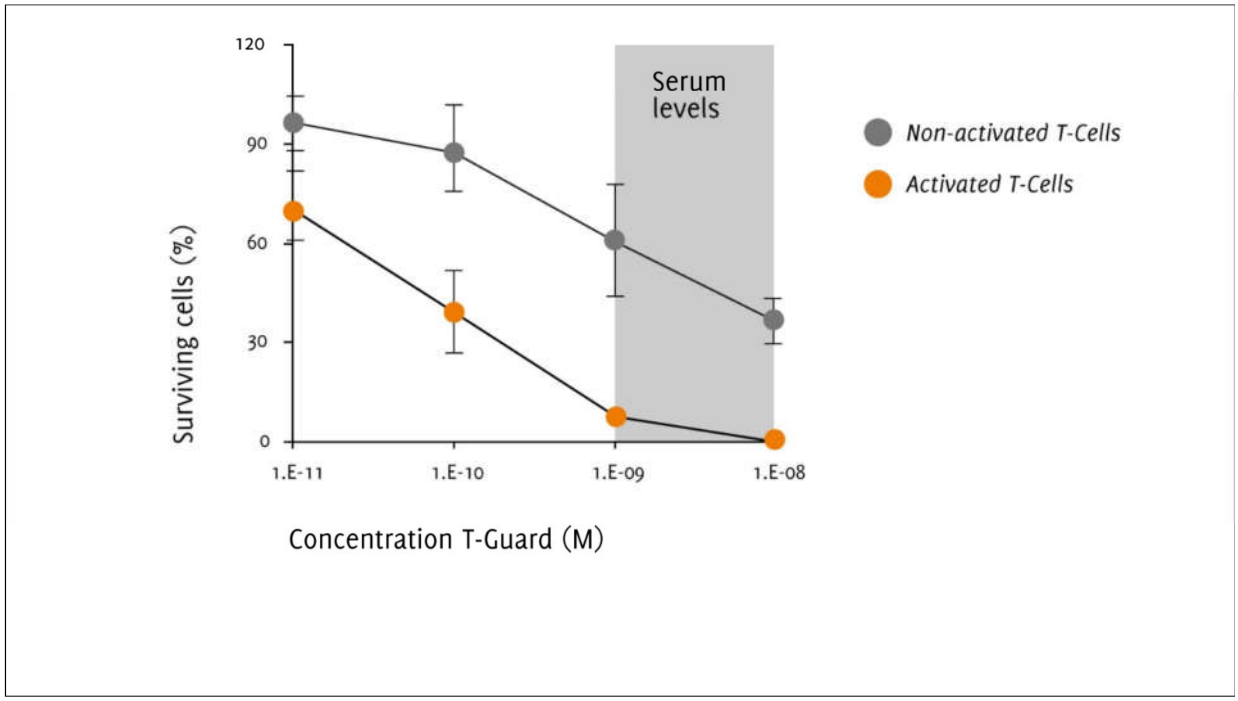
Figure 1-2: Synergistic cell kill by the T-Guard combination



1.3.1.1.3 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.

1.3.1.1.4 NK Cells Apoptosis

Aside from T cells, the immunotoxin-antibody combination WT1-RTA also targets NK cells (CD3-/CD7+). In the Phase 1/2 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 production of cytokines such as Interferon (IFN)-gamma (Gill, Olson et al. 2009).

1.3.1.2 Clinical Data

T-Guard has been evaluated in three 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 1/2 study in 20 patients with SR-aGVHD (Groth, van Groningen et al. 2017). T-Guard was also investigated in a single-arm Phase 3 trial (BMT CTN1802), although this study was closed early. Results from these studies are summarized below.

1.3.1.2.1 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 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 for 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 (see 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).

1.3.1.2.2 T-Guard 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. 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 grade II to IV SR-aGVHD. Seventeen of these patients (85%) had grade III-IV SR-aGVHD, and all 20 patients had visceral involvement; 18 with GI (90%) and 5 with 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%).

1.3.1.2.3 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 medications. Nevertheless, in several patients, a further decrease was observed during the treatment period. According to the Principal Investigators (PIs), thrombocytopenia and hypoalbuminemia were well-manageable. TEAEs from the overall study are outlined in Table 1-1 from the Phase 1/2 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%)
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)	
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%).

1.3.1.2.4 BMT CTN 1802 T-Guard open-label, single-arm Phase 3 clinical trial in SR-aGVHD

A Phase 3 single arm, open-label, multicenter clinical trial to evaluate the safety and efficacy of T-Guard was opened to accrual in November 2018 but was closed after the first three participants died within 30 days due to infection/sepsis. Careful review identified significant risk factors and features prognostic of early death at the start of T-Guard treatment in these 3 patients, including findings concerning for progressive infection, renal dysfunction, prior sirolimus, prior checkpoint inhibitor use, high body mass index, and active or suspected thrombotic microangiopathy (TMA).

A subsequent in-depth analysis compared the nine patients (3 in BMT CTN 1802 and 6 in the prior studies) who died within 30 days of enrollment to the 26 patients who survived more than 30 days in both trials. An important factor identified is the LDH at enrollment being equal or higher than 290 (median 308 vs. 222) with 67% of patients with early death vs. 27% if lower than 289 (p=0.05). An elevated LDH in this setting may be a marker of severe microvascular injury/tissue damage and may thus represent transplant-associated TMA which carries a high risk for imminent death. Therefore, TMA screening with LDH criteria is mandated at screening for proposed trial to mitigate risks to patients on study.

Further, the Body Mass Index (BMI) of the 9 patients with early death is higher (median 28.6 vs. 22.8, p=0.03) than the BMI of those who survived beyond Day 30. While no correlation between the early deaths and BMI or T-Guard exposure could be found, for future trials patients with a

BMI greater than or equal to 35 are excluded and the Body Surface Area (BSA)-dose calculation will be optimized for patients exceeding 125% of their ideal body weight (IBW) as is usual practice for IV treatment (e.g., chemotherapy). More details can be found in section 2.5.1.4 Dose and Administration of T-Guard and in the current version of the Investigators Brochure.

Further analysis of all T-Guard patient outcomes as combined across all studies including Phase 1/2/3 trials and the Expanded Access Program, and inclusive of the three deaths on the BMT CTN 1802 protocol, suggests that T-Guard could provide a new and more effective therapeutic option for patients that currently have very few approved options. The efficacy evaluation now includes 35 SR-aGVHD patients, of whom 15 (42.8%) had a Complete Response (CR) and 6 (17.1%) a Partial Response, yielding an Overall Response Rate (ORR) of 60.0%, with a 6-month OS of 60% (including the BMT CTN 1802 participants). These results compared favorably with higher Day 28 CR rate (historical control of 34.4%), similar to the ORR of 62.3%, and higher than the 6-month OS as reported in the REACH2 trial of 48.7% (Zeiser, NEJM 2020). The high D28 CR rate with T-Guard appears to be especially (or particularly) promising as CR at D28 is the strongest correlate with long term survival. Thus, in consultation with the FDA, BMT CTN 1802 was closed to pursue the randomized clinical trial design with ruxolitinib in Grade III-IV SR-aGVHD to be studied in current trial. The experience from BMT CTN 1802 has helped clarify inclusion/exclusion criteria and safety monitoring for this randomized trial. The randomization may also help determine if the toxicities observed in the single arm study may have been associated with T-Guard administration or if they are inherent to the very high-risk population eligible for SR-aGVHD trials.

1.3.2 Ruxolitinib

Ruxolitinib, a kinase inhibitor, inhibits JAK1 and JAK2 which mediate the signaling of a number of cytokines and growth factors that are important for hematopoiesis and immune function. JAK signaling involves recruitment of STATs (signal transducers and activators of transcription) to cytokine receptors, activation and subsequent localization of STATs to the nucleus leading to modulation of gene expression.

1.3.2.1 Clinical Data

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

The safety of ruxolitinib was assessed in 617 patients in six clinical studies with a median duration of follow-up of 10.9 months, including 301 patients with myelofibrosis in two Phase 3 studies.

In these myelofibrosis studies, patients had a median duration of exposure to ruxolitinib of 9.5 months (range 0.5 to 17 months), with 88.7% of patients treated for more than 6 months and 24.6% treated for more than 12 months. One hundred and eleven (111) patients started treatment at 15 mg twice daily and 190 patients started at 20 mg twice daily. In patients starting treatment with 15 mg twice daily (pretreatment platelet counts of 100 to 200 x 109/L) and 20 mg twice daily (pretreatment platelet counts greater than 200 x 109/L), 65% and 25% of patients,

respectively, required a dose reduction below the starting dose within the first 8 weeks of therapy.

In the SR-aGVHD setting, a multicenter, randomized, open-label Phase 3 study of ruxolitinib versus BAT was performed (REACH2). Of the 154 patients assigned to the ruxolitinib arm, 152 were treated with ruxolitinib, of the 155 patients assigned to the control arm, 150 patients received BAT.

The most common adverse events up to day 28 were thrombocytopenia (in 50 of 152 patients [33%] receiving ruxolitinib and 27 of 150 [18%] patients receiving the control), anemia (in 46 [30%] and 42 [28%], respectively), and CMV infection (in 39 [26%] and 31 [21%]) (Zeiser, Bubnoff et al. 2020).

Treatment discontinuation occurred in 111 of 154 patients (72%) assigned to ruxolitinib and in 132 of 155 (85%) assigned to the control; the most common reason was lack of efficacy (in 32 [21%] and 68 [44%], respectively). The median duration of exposure to therapy was 63 days (range, 6 to 396) in the ruxolitinib group and 29 days (range, 1 to 188) in the control group. The median dose intensity of ruxolitinib was 16.8 mg per day (interquartile range, 11.9 to 19.6).

Table 1-3 presents the most common adverse reactions occurring in the 152 patients who received ruxolitinib and the 150 patients treated with the control in the randomized REACH2 study.

Table 1-3: Most Frequent Adverse Events up to Day 28 (Safety Population)*

Most Frequent Adverse Events up to Day 28 (Safety Population)*				
Event	**Ruxolitinib (N= 152)**		**Control (N=150)**	
	Any Grade	Grade ≥ 3	Any Grade	Grade ≥ 3
	number of patients (percent)			
Any adverse event	145 (95)	118 (78)	140 (93)	117 (78)
Thrombocytopenia	50 (33)	41 (27)	27 (18)	23 (15)
Anemia	46 (30)	33 (22)	42 (28)	28 (19)
Cytomegalovirus infection†	39 (26)	11 (7)	31 (21)	12 (8)
Peripheral edema	28 (18)	2 (1)	26 (17)	1 (1)
Platelet count decreased	26 (17)	22 (14)	21 (14)	20 (13)
Neutropenia	24 (16)	20 (13)	19 (13)	14 (9)
Hypokalemia	20 (13)	9 (6)	25 (17)	9 (6)
Hypertension	16 (11)	9 (6)	14 (9)	6 (4)
Hypoalbuminemia	16 (11)	6 (4)	15 (10)	10 (7)
Pyrexia	16 (11)	2 (1)	17 (11)	2 (1)
Hypomagnesemia	15 (10)	0	20 (13)	1 (1)
Diarrhea	14 (9)	7 (5)	15 (10)	5 (3)
White-cell count decreased	14 (9)	11 (7)	13 (9)	11 (7)
Nausea	13 (9)	0	9 (6)	0
Hypocalcemia	12 (8)	3 (2)	10 (7)	4 (3)
Hypophosphatemia	12 (8)	5 (3)	14 (9)	7 (5)
Abdominal pain	11 (7)	4 (3)	7 (5)	2 (1)
Sepsis	11 (7)	10 (7)	6 (4)	5 (3)
Acute kidney injury	10 (7)	1 (1)	3 (2)	3 (2)
Alanine aminotransferase increased	10 (7)	3 (2)	10 (7)	4 (3)
Neutrophil count decreased	10 (7)	10 (7)	14 (9)	11 (7)
Vomiting	10 (7)	1 (1)	6 (4)	0
Epstein-Barr virus infection	9 (6)	0	8 (5)	3 (2)
Hyperglycemia	9 (6)	5 (3)	14 (9)	8 (5)
Hypogammaglobulinemia	9 (6)	2 (1)	5 (3)	0
Fall	8 (5)	1 (1)	1 (1)	0
Hyperkalemia	8 (5)	3 (2)	6 (4)	2 (1)
Hypotension	8 (5)	4 (3)	9 (6)	3 (2)
Leukopenia	8 (5)	7 (5)	2 (1)	2 (1)
Pancytopenia	8 (5)	7 (5)	6 (4)	5 (3)
Urinary tract infection	8 (5)	3 (2)	6 (4)	4 (3)
Gamma-glutamyltransferase increased	7 (5)	3 (2)	10 (7)	7 (5)
Pneumonia	6 (4)	5 (3)	8 (5)	7 (5)
Blood bilirubin increased	5 (3)	3 (2)	12 (8)	7 (5)
Pain in extremity	4 (3)	2 (1)	8 (5)	1 (1)

*Shown are the adverse events that had an incidence of at least 5% in either group. The safety population included all patients who received at least on dose of trial therapy.

†A distinction between cytomegalovirus infection and reactivation was not made in this trial.

1.3.2.1.1 Thorough QT Study

The effect of single dose ruxolitinib 25 mg and 200 mg on QTc interval was evaluated in a randomized, placebo-, and active-controlled (moxifloxacin 400 mg) four-period crossover thorough QT study in 47 healthy subjects. In a study with demonstrated ability to detect small effects, the upper bound of the one-sided 95% confidence interval for the largest placebo adjusted, baseline-corrected QTc based on Fridericia correction method (QTcF) was below 10ms, the threshold for regulatory concern. The dose of 200 mg is adequate to represent the high exposure clinical scenario.

1.3.2.1.2 Drug Interactions - CYP3A4 inhibitors

Strong CYP3A4 inhibitors: In a trial of 16 healthy volunteers, a single dose of 10 mg of ruxolitinib was administered alone on Day 1 and a single dose of 10 mg of ruxolitinib was administered on Day 5 in combination with 200 mg of ketoconazole (a strong CYP3A4 inhibitor, given twice daily on Days 2 to 5). Ketoconazole increased ruxolitinib C_{max} and AUC by 33% and 91%, respectively. Ketoconazole also prolonged ruxolitinib half-life from 3.7 to 6.0 hours.

Fluconazole: Simulations using physiologically-based pharmacokinetic (PBPK) models suggested that fluconazole (a dual CYP3A4 and CYP2C9 inhibitor) increases steady state ruxolitinib AUC by approximately 100% to 300% following concomitant administration of 10 mg of Jakafi twice daily with 100 mg to 400 mg of fluconazole once daily, respectively.

Mild or moderate CYP3A4 inhibitors: In a trial of 15 healthy volunteers, a single dose of 10 mg of ruxolitinib was administered alone on Day 1 and a single dose of 10 mg of Jakafi was administered on Day 5 in combination with 500 mg of erythromycin (a moderate CYP3A4 inhibitor, given twice daily on Days 2 to 5). Erythromycin increased ruxolitinib C_{max} and AUC by 8% and 27%, respectively.

1.3.2.1.3 Drug Interactions - CYP3A4 inducers

In a trial of 12 healthy volunteers, a single dose of 50 mg of Jakafi was administered alone on Day 1 and a single dose of 50 mg of Jakafi was administered on Day 13 in combination with 600 mg of rifampin (a strong CYP3A4 inducer, given once daily on Days 3 to 13). Rifampin decreased ruxolitinib C_{max} and AUC by 32% and 61%, respectively. In addition, the relative exposure to ruxolitinib's active metabolites increased approximately 100%.

1.3.2.1.4 Pharmacodynamics

Ruxolitinib inhibits cytokine induced STAT3 phosphorylation in whole blood from healthy subjects and MF patients. Jakafi administration resulted in maximal inhibition of STAT3 phosphorylation 2 hours after dosing which returned to near baseline by 10 hours in both healthy subjects and myelofibrosis patients.

1.3.2.1.5 Pharmacokinetics

Absorption: In clinical studies, ruxolitinib is rapidly absorbed after oral Jakafi administration with maximal plasma concentration (C_{max}) achieved within 1 to 2 hours post-dose. Based on a mass balance study in humans, oral absorption of ruxolitinib was estimated to be at least 95%. Mean ruxolitinib C_{max} and total exposure (AUC) increased proportionally over a single dose range of 5

to 200 mg. There were no clinically relevant changes in the pharmacokinetics of ruxolitinib upon administration of Jakafi with a high-fat meal, with the mean C_{max} moderately decreased (24%) and the mean AUC nearly unchanged (4% increase). PK data in transplant patients showed lower absorption (decreased C_{max} and AUC) but similar elimination half-life compared to published data from healthy volunteers. (Ali, H., Snyder, D., Stiller, T., et al 2019)

Distribution: The apparent volume of distribution of ruxolitinib at steady state is 53 to 65 L in myelofibrosis patients. Binding to plasma proteins *in vitro* is approximately 97%, mostly to albumin.

Metabolism: In vitro studies suggest that ruxolitinib is metabolized by CYP3A4 and to a lesser extent by CYP2C9.

Elimination: Following a single oral dose of [14C]-labeled ruxolitinib in healthy adult subjects, elimination was predominately through metabolism with 74% of radioactivity excreted in urine and 22% excretion via feces. Unchanged drug accounted for less than 1% of the excreted total radioactivity. The mean elimination half-life of ruxolitinib is approximately 3 hours and the mean half-life of ruxolitinib + metabolites is approximately 5.8 hours.

Effects of Age, Gender, or Race: In healthy subjects, no significant differences in ruxolitinib pharmacokinetics were observed with regard to gender and race. In a population pharmacokinetic evaluation in myelofibrosis 16 patients, no relationship was apparent between oral clearance and patient age or race, and in women, clearance was 17.7 L/h and in men, 22.1 L/h with 39% inter-subject variability.

1.3.2.2 Toxicology

Ruxolitinib was not carcinogenic in the 6-month Tg.rasH2 transgenic mouse model or in a 2-year carcinogenicity study in the rat.

Ruxolitinib was not mutagenic in a bacterial mutagenicity assay (Ames test) or clastogenic in *in vitro* chromosomal aberration assay (cultured human peripheral blood lymphocytes) or *in vivo* in a rat bone marrow micronucleus assay.

In a fertility study, ruxolitinib was administered to male rats prior to and throughout mating and to female rats prior to mating and up to the implantation day (gestation day 7). Ruxolitinib had no effect on fertility or reproductive function in male or female rats at doses of 10, 30 or 60 mg/kg/day. However, in female rats' doses of greater than or equal to 30 mg/kg/day resulted in increased post-implantation loss. The exposure (AUC) at the dose of 30 mg/kg/day is approximately 34% the clinical exposure at the maximum recommended dose of 25 mg twice daily.

1.3.2.3 In vitro studies

In vitro, ruxolitinib and its M18 metabolite do not inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 or CYP3A4. Ruxolitinib is not an inducer of CYP1A2, CYP2B6 or CYP3A4 at clinically relevant concentrations.

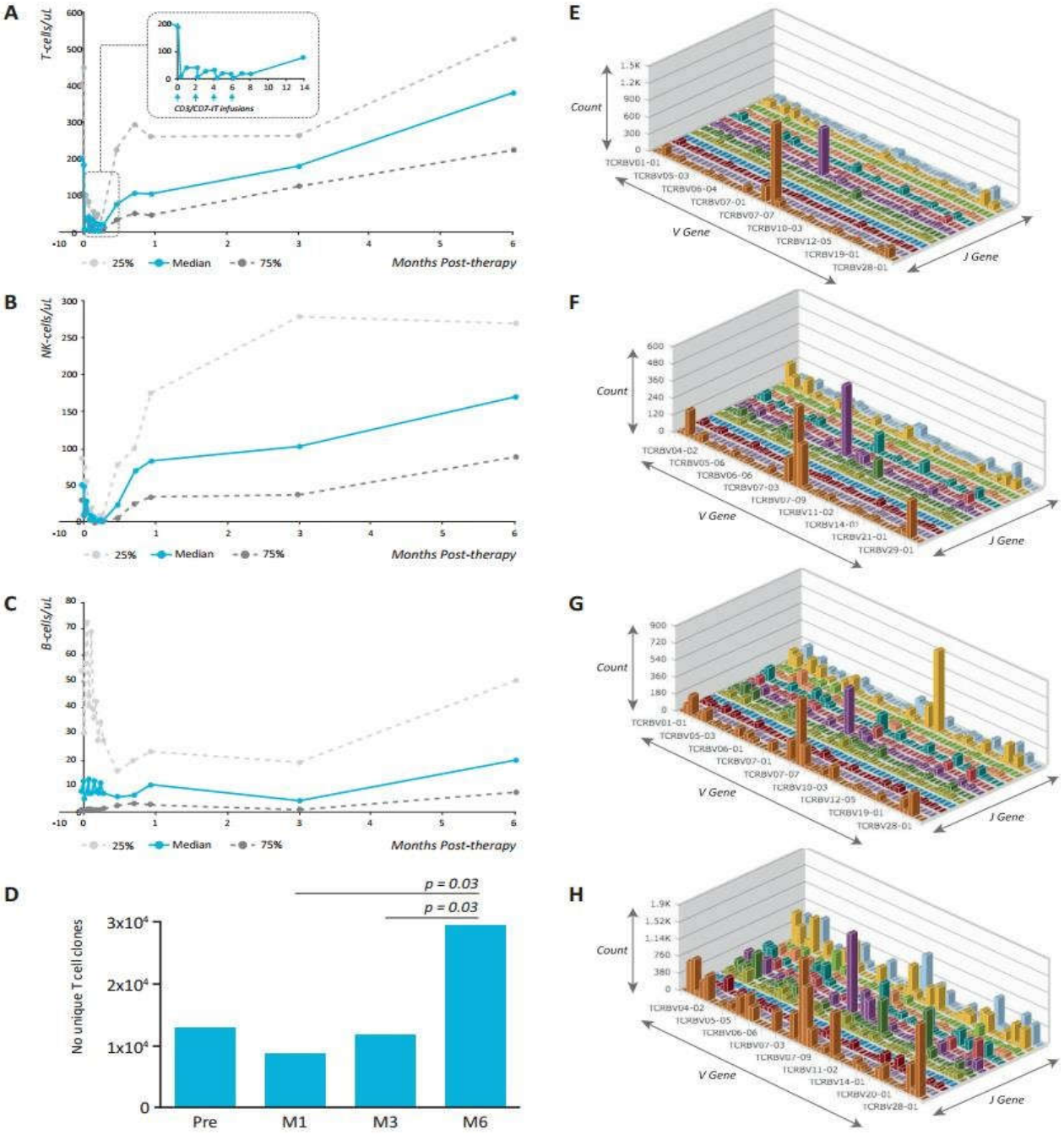
In vitro, ruxolitinib and its M18 metabolite do not inhibit the P-gp, BCRP, OATP1B1, OATP1B3, OCT1, OCT2, OAT1 or OAT3 transport systems at clinically relevant concentrations. Ruxolitinib is not a substrate for the P-gp transporter.

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 (see 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.

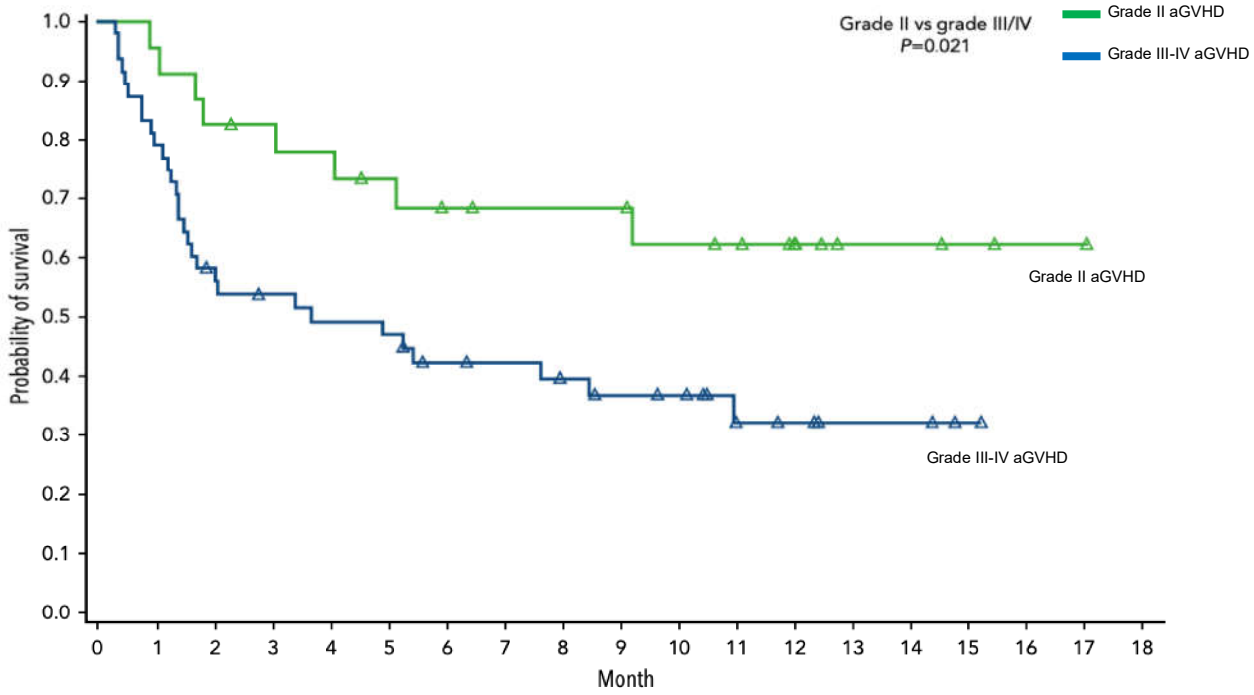
Figure 1-4: T-Guard Observed Immune Reconstitution



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.

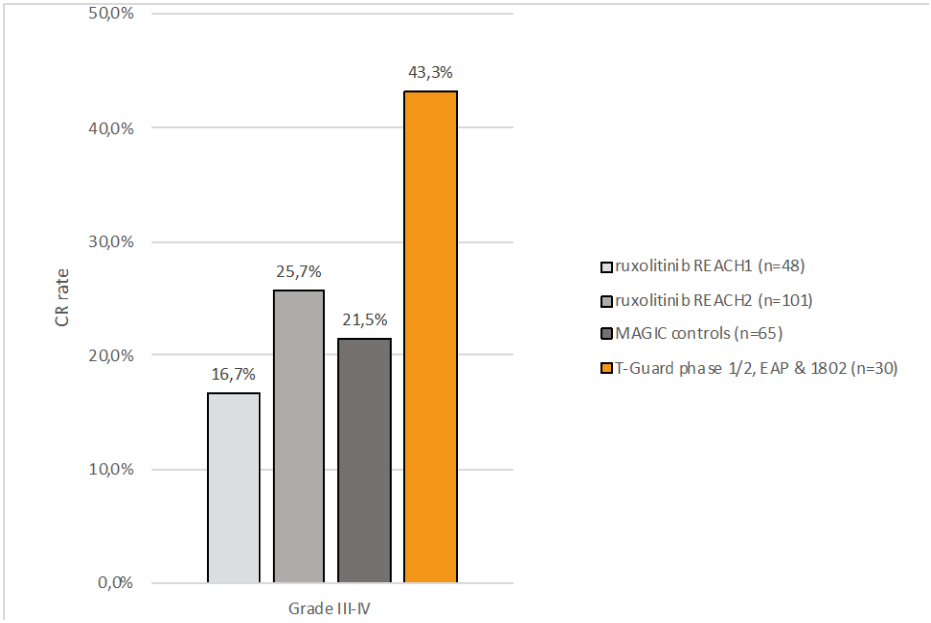
In this study, ruxolitinib will be the comparator drug since ruxolitinib is the only currently FDA approved drug for this indication, and in the EU, it is broadly used based on the REACH2 study demonstrating superiority over BAT. It is important to note that the ORR and benefits of ruxolitinib therapy are primarily driven by responses in patients with Grade II SR-aGVHD. Patients treated with ruxolitinib for grade III-IV SR-GVHD have significantly worse survival (Figure 1-5). An ad-hoc analysis of available data, of treatment outcomes for patients with Grade III-IV SR-aGVHD showed that patients treated with T-Guard had the highest Day 28 CR (43.3%) followed by patients treated with ruxolitinib on REACH2 (25.7%). The CR rates for patients with Grade III-IV SR-GVHD treated with ruxolitinib on REACH1 or BAT on the MAGIC natural history trial are also shown for comparison (Figure 1-6). The higher CR rate seen with T-Guard is consistent with a higher OS observed in patients treated with T-Guard (Figure 1-7). Taken together the preliminary data and ad hoc analyses suggest that T-Guard may be efficacious for Grade III-IV SR-aGVHD and compares favorably to existing treatments. In this Phase 3 randomized, open-label, multicenter study we will directly compare T-Guard to ruxolitinib as treatment for Grade III-IV SR-aGVHD, the group at highest risk for treatment failure and death.

Figure 1-5: Overall Survival Grade II vs. Grade III-IV SR-aGVHD Ruxolitinib



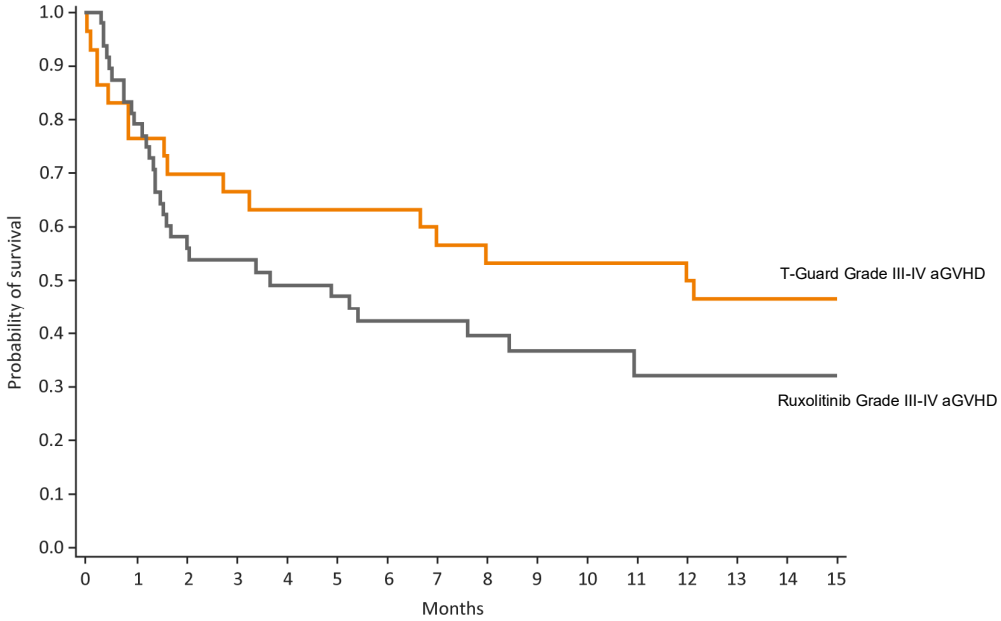
OS by aGVHD grade at enrollment. aGVHD grade at enrollment was significantly associated with OS by log-rank test in the Kaplan-Meier analysis (P = .021). In model-based analysis of OS by Cox regression, aGVHD grade III/IV was again significantly associated with reduced OS (aGVHD grade III/IV vs. grade II; HR, 0.334; 95% CI, 0.150-0.747; P = .0076) (Jagasia et al. 2020).

Figure 1-6: Day 28 CR rates Grade III-IV SR-aGVHD patients T-Guard compared to data from ruxolitinib and MAGIC controls



Phase 1/2 study (n=17; (Groth et al. 2019)), expanded access program (n=11; (Groningen et al. 2019)) and BMT CNT 1802 (n=2)).

Figure 1-7: Overall survival Grade III-IV SR-aGVHD patients T-Guard vs. ruxolitinib



T-Guard integrated survival curve of Grade III-IV patients (n=30; data on file) vs. REACH1 (Jagasia et al. 2020)

CHAPTER 2

2 STUDY DESIGN

2.1 STUDY OVERVIEW

This is a Phase 3, open-label, randomized, international multicenter trial designed to compare T-Guard to ruxolitinib for the treatment of patients with Grade III or IV 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 response (CR) rate in patients with Grades III and IV SR-aGVHD compared to ruxolitinib.

2.3 STUDY OBJECTIVES

2.3.1 Primary Objective

The primary objective of this trial is to assess the rate of CR on Day 28 post-randomization in Grades III and IV SR-aGVHD patients treated with T-Guard treatment in comparison to ruxolitinib.

2.3.2 Secondary Objectives

1. Estimate overall survival (OS) at Days 60, 90 and 180 post-randomization.
2. Evaluate the duration of complete response (DoCR).
3. Estimate the time to CR from randomization.
4. Estimate the overall response rate (CR or partial response (PR)) at Days 14, 28, and 56 post-randomization.
5. Describe proportions of CR, PR, mixed response (MR), no response (NR), and progression of aGVHD at Days 6, 14, 28, and 56 post-randomization.
6. Estimate the cumulative incidence of NRM at Days 100 and 180 post-randomization.
7. Estimate relapse-free survival at Day 180 post-randomization.
8. Estimate GVHD-free survival at Days 90 and 180 post-randomization.
9. Estimate the cumulative incidence of cGVHD at Day 180 post-randomization.
10. Estimate the cumulative incidence of underlying disease relapse/progression at Day 180 post-randomization.
11. Describe the incidence of infections.
12. Describe the incidence of toxicities.
13. Assess the pharmacokinetics of T-Guard.
14. Assess the immunogenicity of T-Guard.

2.3.3 Exploratory Objectives

1. Describe the proportion of participant free of systemic steroids by Day 180 post-randomization.
2. Estimate the incidence of CMV reactivation requiring treatment by Day 180 post-randomization.
3. Estimate the incidence of EBV-associated lymphoproliferative disorder or EBV reactivation requiring treatment with rituximab by Day 180 post randomization.
4. Evaluate the evolution and characteristics of specific cell populations at randomization and at Days 0, 14, 28, 56 and 180.
5. Evaluate aGVHD Biomarkers at baseline and at Days 6, 14 and 28 post-randomization.
6. Describe changes in patient-reported outcomes (PROs) from baseline to Days 28, 90 and 180 post-randomization.
7. Estimate incidence of TMA at Days 6, 14, 21 and 28 post-randomization.
8. Describe the EASIX score at screening.

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. Patients must be at least 18.0 years of age at the time of consent.
2. Patient has undergone first allo-HSCT from any donor source or graft source. Recipients of nonmyeloablative, reduced intensity, and myeloablative conditioning regimens are eligible.
3. Patients diagnosed with Grade III/IV SR-aGVHD after allo-HSCT. SR includes aGVHD initially treated at a lower steroid dose, but must meet one of the following criteria:
   - Progressed or new organ involvement after 3 days of treatment with methylprednisolone (or equivalent) of greater than or equal to 2 mg/kg/day
   - No improvement after 7 days of primary treatment with methylprednisolone (or equivalent) of greater than or equal to 2mg/kg/day
   - Patients with visceral (GI and/or liver) plus skin aGVHD at methylprednisolone (or equivalent) initiation with improvement in skin GVHD without any improvement in visceral GVHD after 7 days of primary treatment with methylprednisolone (or equivalent) of greater than or equal to 2mg/kg/day
   - Patients who have skin GVHD alone and develop visceral aGVHD during treatment with methylprednisolone (or equivalent) of greater than or equal to 1mg/kg/day and do not improve after 3 days of greater than or equal to 2mg/kg/day

   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 methylprednisolone (or equivalent) treatment. Staging is performed per MAGIC criteria (see APPENDIX C).
4. Patients must 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.
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.

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 a creatinine greater than or equal to 2mg/dL or estimated creatinine clearance less than 40 mL/min or those requiring hemodialysis.
2. Patients who have been diagnosed with active TMA, defined as meeting **all** the following criteria:
   - Greater than 4% schistocytes in blood (or equivalent if semiquantitative scale is used e.g., 3+ or 4+ schistocytes on peripheral blood smear)
   - De novo, prolonged or progressive thrombocytopenia (platelet count less than 50 x 10^9/L or 50% or greater reduction from previous counts)
   - Sudden and persistent increase in lactate dehydrogenase concentration greater than 2x ULN
   - Decrease in hemoglobin concentration or increased transfusion requirement attributed to Coombs-negative hemolysis
   - Decrease in serum haptoglobin
3. Patients who have previously received treatment with eculizumab.
4. Patients who have previously received checkpoint inhibitors (either before or after allo-HCT).
5. Patients who have been diagnosed with overlap syndrome, that is, with any concurrent features of cGVHD.
6. Patients requiring mechanical ventilation or vasopressor support.
7. Patients who have received any systemic treatment, besides steroids, as upfront treatment of aGVHD or as treatment for SR-aGVHD. Reinstitution of previously used GVHD prophylaxis agents (e.g., tacrolimus, cyclosporin, MTX, MMF) or substitutes in cases with previously documented intolerance will be permitted. Previous treatment with a JAK inhibitor as part of GVHD prophylaxis or treatment is not allowed.
8. Patients who have severe hypoalbuminemia, with an albumin of less than or equal to 1 g/dl.
9. Patients who have a creatine kinase (CK) level of greater than 5 times the upper limit of normal.
10. 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. Persisting fever without other signs or symptoms will not be interpreted as progressing infection. Progression of infection is defined as:
    - hemodynamic instability attributable to sepsis OR
    - new symptoms attributable to infection OR
    - worsening physical signs attributable to infection OR
    - worsening radiographic findings attributable to infection

    Patients with radiographic findings attributable to infection within 4 weeks prior to enrollment must have a repeat radiographic exam within one week of enrollment that documents absence of worsening.
11. Patients with evidence of relapsed, progressing, or persistent malignancy, or who have been

treated for relapse after transplant, or who may require rapid immune suppression withdrawal as pre-emergent treatment of early malignancy relapse.

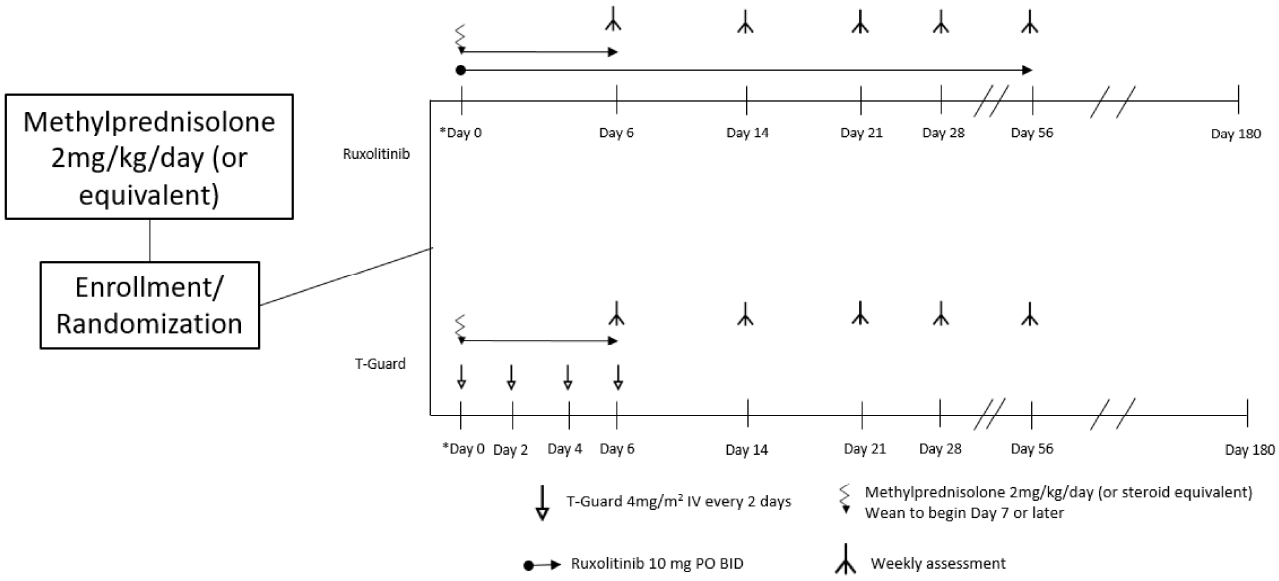
12. Patients with evidence of minimal residual disease requiring withdrawal of systemic immune suppression.
13. Patients with unresolved serious toxicity or complications (other than aGVHD) due to previous transplant.
14. History of sinusoidal obstruction syndrome (SOS)/veno-occlusive disease (VOD).
15. Patients with known hypersensitivity to any of the components murine mAb or Recombinant Ricin Toxin A-chain (RTA).
16. Patients who have had treatment with any other investigational agent, device, or procedure within 21 days (or 5 half-lives, whichever is greater) prior to enrollment. An investigational agent is defined as medications without any known FDA or EMA approved indications.
17. Patients who have received more than one allo-HSCT.
18. Patients with known human immunodeficiency virus infection.
19. Patients who have a BMI greater than or equal to 35 kg/m^2.
20. Patients who are taking sirolimus **must** discontinue prior to starting study treatment.

    The sirolimus blood level must be less than 2 ng/mL prior to starting study treatment.
21. 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 study treatment.
22. 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 study treatment.
23. 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.
24. 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

Participants should start treatment as close to the time of randomization as possible but no later than 72 hours after randomization.

Figure 2-1: Treatment Schema



2.5.1 T-Guard

2.5.1.1 Drug Information for T-Guard

T-Guard is a fixed dose combination of two active ingredients, SPV-T3a-RTA and WT1-RTA. It will be supplied as a single, frozen liquid for infusion and needs to be stored at -20°C (± 5°C) under controlled conditions.

T-Guard is delivered as a pack containing two 50 RDIN vials of type-I glass with a rubber stopper and an aluminum flip-off cap. Each vial contains a fixed dose combination of 2.5 mg purified SPV-T3a-RTA and 2.5 mg purified WT1-RTA in a fill volume of 25 mL/vial at a concentration of 0.2 mg protein/mL.

Stability testing of the infusion concentrate is ongoing, and no definite shelf life can be proposed yet. The clinical batches will continue to be validated to justify the retest dates by means of real time stability data according to a pre-defined stability protocol.

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.

2.5.1.2 Storage, Handling, and Dispensing of T-Guard

T-Guard vials are to be stored at -20°C (± 5°C) until use. T-Guard will be prepared for infusion by the pharmacy of the participating centers.

A certificate of analysis will be provided indicating the expiry date.

Before administration to the participant, the medication is brought to room temperature for 1.5 hours, 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°C ± 5°C), in a controlled environment.

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

2.5.1.3 Drug Accountability for T-Guard

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 drug 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 participant. This record should contain which supplies are issued to which participant, 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 drug should be kept and added to the drug accountability record. All study drug in these categories will be inventoried by the Clinical Research Associate (CRA) during and at the conclusion of the study. The CRA will arrange for their secure disposal at the end of the study.

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

2.5.1.4 Dose and Administration of T-Guard

It is strongly recommended all doses of T-Guard be received while the participant is hospitalized. T-Guard will be administered over 4 hours every 2 calendar days on Days 0, 2, 4, and 6, at a dose of 4mg/m^2 BSA (not to exceed a dose of 10mg). Dosing should be per the following steps:

- Measure the participant's actual body weight (ABW)
- Determine the ideal bodyweight
    - Males IBW = 50kg + 2.3kg for each inch over 5 feet (or for each 2.5cm over 1.52m)
    - Females IBW = 45.5kg + 2.3kg for each inch over 5 feet (or for each 2.5cm over 1.52m)
- When the participant's body weight is more than 125% of their IBW, the BSA calculation is based on the adjusted ideal body weight (AIBW; formula 2) as is usual practice for IV treatment (e.g. chemotherapy).

  For participants weighing more than 125% of their Ideal Body Weight, calculate the Adjusted Ideal Body Weight: AIBW = IBW + [(0.25) x (ABW - IBW)]

- Use the (adjusted ideal) body weight to determine BSA per the Mosteller calculation

$$BSA(m^2) = \sqrt{\frac{HEIGHT(cm) \times WEIGHT(kg)}{3600}}$$

- Multiply BSA with 4mg/m^2 as the T-Guard dose. If the participant's BSA is more than 2.5m^2, the dose calculation should use 2.5m^2. This dose will be maintained for all infusions (dose adjustments for changes in weight during the treatment period will not be made).

Premedication with antihistaminica (e.g., diphenhydramine 50mg by mouth or 25mg IV, clemastine 2mg IV) is strongly recommended,20-60 minutes prior to each dose. 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 should 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 participant 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.

Participants should receive a maximum of 4 doses. The minimal interval between doses is 2 days (the start of infusion should be no less than 40 hours from the start of the previous infusion). If toxicity occurs, then dosing should be delayed until toxicity improves, but all dosing must be completed within 14 days. Infusions of T-Guard that exceed 4 hours are not considered deviations. Pauses in the infusion due to a participant reaction need to be documented.

2.5.1.5 T-Guard Infusion Delays

In the event dosing must be delayed due to occurrence of toxicity, the protocol allows up to 14 days to receive all four infusions. If the infusion visits are delayed, then the assessments for each infusion should still be done.

If an infusion is delayed to Day 11-13, the Day 14 visit can be combined as appropriate respecting the 3-day window for the Day 14 visits. However, it's ideal for the assessments to be completed on Day 14. 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 investigator discretion. If all assessments for the infusion (Day 4 or 6) and Day 14 are combined to be completed on the same day, Peripheral blood for anti-drug-antibodies (ADA), Peripheral blood for GVHD biomarkers, and a urine sample for GVHD biomarkers are to be collected prior to infusion. If the samples can't be drawn pre-infusion, they may be collected the day after.

Centers 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.

2.5.1.6 Toxicities and Guidelines for Withholding T-Guard

A physician must be available for all infusions in order to treat anaphylaxis or CRS, should it occur. 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 mAb including urticaria, bronchospasm, anaphylaxis, Arthurs 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 per institutional standards, 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 participants 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. Participants 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 regardless of the albumin level, then further dosing can be discussed with the protocol chairs.

Myalgia and serum CK-elevation:

There is an association of myositis with Ricin-based toxicity. Therefore, participants should be monitored for signs of myositis. If participants 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 participant.

Other toxicities:

Participants 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:

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

2.5.1.7 T-Guard Discontinuation

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

2.5.1.8 aGVHD Progression or Non-response

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

2.5.2 Ruxolitinib

2.5.2.1 Drug Information for Ruxolitinib

Ruxolitinib phosphate is a kinase inhibitor with the chemical name (*R*)-3-(4-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)-1*H*-pyrazol-1-yl)-3-cyclopentylpropanenitrile phosphate and a molecular weight of 404.36.

Ruxolitinib phosphate is a white to off-white to light pink powder and is soluble in aqueous buffers across a pH range of 1 to 8.

Jakafi (ruxolitinib) Tablets are for oral administration. Each tablet contains ruxolitinib phosphate equivalent to 10 mg or 5 mg, of ruxolitinib free base together with microcrystalline cellulose,

lactose monohydrate, magnesium stearate, colloidal silicon dioxide, sodium starch glycolate, povidone and hydroxypropyl cellulose.

2.5.2.2 Drug Supply of Ruxolitinib

Ruxolitinib will be provided through Day 56 for all participants. For centers in the United States, commercial supply of ruxolitinib will be utilized, covered for by the Sponsor until Day 56, with relabeling of medication per the Pharmacy Manual. Participants continuing beyond Day 56 will receive standard commercial supply. For centers within Europe, ruxolitinib will be provided by the Sponsor, labeled for this protocol, through the site pharmacy and until study treatment discontinuation (e.g., due to treatment failure, relapse, study withdrawal, death) or transformation into cGVHD.

2.5.2.3 Dose and Administration of Ruxolitinib

Participants randomized to the ruxolitinib arm will receive 10mg orally twice daily to begin on Day 0. Dose modifications for toxicity are described below. Participants responding to treatment may be tapered off ruxolitinib as needed, starting no earlier than Day 56. The dose tapering strategy should be based on evaluation of the condition of the participant, current dosing regimen and the clinical judgment of the Investigator.

2.5.2.4 Toxicities and Guidelines for Withholding Ruxolitinib

The following are expected toxicities associated with ruxolitinib.

Myelosuppression:

Treatment with ruxolitinib can cause thrombocytopenia, anemia and neutropenia.

Manage thrombocytopenia by reducing the dose or temporarily interrupting ruxolitinib. Platelet transfusions may be necessary.

Participants developing anemia may require blood transfusions and/or dose modifications of ruxolitinib. Severe neutropenia (ANC less than 0.5 X 10^9/L) was generally reversible by withholding ruxolitinib until recovery. Perform a pre-treatment complete blood count (CBC) and monitor CBCs every 2 to 4 weeks until doses are stabilized, and then as clinically indicated.

Risk of Infection:

Serious bacterial, mycobacterial, fungal and viral infections have occurred. Delay starting therapy with ruxolitinib until active serious infections have resolved. Observe participants receiving ruxolitinib for signs and symptoms of infection and manage promptly.

Tuberculosis: Tuberculosis infection has been reported in participants receiving Jakafi. Observe participants receiving ruxolitinib for signs and symptoms of active tuberculosis and manage promptly. Prior to initiating ruxolitinib, participants should be evaluated for tuberculosis risk factors, and those at higher risk should be tested for latent infection. Risk factors include, but are not limited to, prior residence in or travel to countries with a high prevalence of tuberculosis, close contact with a person with active tuberculosis, and a history of active or latent tuberculosis where an adequate course of treatment cannot be confirmed. For participants with evidence of active or latent tuberculosis, consult a physician with expertise in the treatment of tuberculosis

before starting ruxolitinib. The decision to continue ruxolitinib during treatment of active tuberculosis should be based on the overall risk-benefit determination.

PML: Progressive multifocal leukoencephalopathy (PML) has occurred with ruxolitinib treatment for myelofibrosis. If PML is suspected, stop ruxolitinib and evaluate.

Herpes Zoster: Advise participants about early signs and symptoms of herpes zoster and to seek treatment as early as possible if suspected.

2.5.2.5 Dose Modifications for Ruxolitinib

Ruxolitinib dose will be adjusted for toxicity. Dose modification guidelines are based on the occurrence and intensity of specific adverse events that are suspected to be drug related. No dose reduction of ruxolitinib starting dose is required for concomitant strong CYP3A4 inhibitors/dual inhibitors. The most relevant information has been provided in Table 2-1. Table 2-2 provides dose reduction steps for ruxolitinib dosing.

Table 2-1: Required Treatment Modification Guidelines for Ruxolitinib-related Toxicities

Grade by NCI CTCAE#[1]	Action[2]
Neutrophil count decreased	**Grade 3**: Decrease 1 dose level (see Table 2-2), monitor ANC daily until resolved to ≤ Grade 2, then resume initial dose level
	Grade 4: Hold dose, monitor ANC daily until resolved until ≤ Grade 3, the resume at next lower dose level. If resolves to ≤ Grade 2, can resume initial dose level. If not resolved in ≤ 14 days while holding, drug must be discontinued.
Febrile neutropenia	**Grade 3**: Hold dose until resolved, then restart at next lower dose level.
Thrombocytopenia	**Grade 4**: (plt <,20,000 – 15,000/mm^3): Decrease 1 dose level until resolved to ≥ 20,000/mm^3. If resolved in ≤ 7 days, then resume initial dose level. If resolved in > 7 days, then maintain the decreased dose.
	Grade 4: (plt <,15,000/mm^3): Hold dose until resolved to ≥ 20,000/mm^3. If resolved to ≤ Grade 3, can resume initial dose level. If not resolved in ≤ 14 days while holding, drug must be discontinued.
Serum creatinine	**Grade 2**: Decrease 1 dose level until resolved to ≤ Grade 1 or baseline, then resume initial dose level
	Grade 3: Hold dose until resolved to ≤ Grade 2, then restart at next lower dose level. If resolves to ≤ Grade 1 can resume initial dose level.
	Grade 4: Discontinue ruxolitinib.
Total bilirubin increased	**Grade 3**: (> 3.0 – 5.0 x ULN): Decrease 1 dose level until resolved to ≤ 3.0 x ULN. Monitor LFTs at least weekly, until resolved to ≤ 3.0 x ULN. If resolved ≤ 14 days, then resume initial dose level. If resolved in > 14 days, then maintain the decreased dose level.
	Grade 3: (>5.0 – 10.0 x ULN): Hold dose. Monitor LFTs at least weekly until resolved to ≤ 3.0 x ULN. If resolved ≤ 14 days, then resume same dose level. If resolved in > 14 days, then restart at the next lower dose level.
	Grade 4: Hold dose. Monitor LFTs at least weekly until resolved to ≤ 3.0 x ULN. If resolved ≤ 14 days, then resume at the next lower dose level. If resolved in > 14 days, then drug must be discontinued. LFTs should be monitored at least weekly until total bilirubin has resolved to baseline or stabilized over 4 weeks.

Grade by NCI CTCAE#[1]	Action[2]
ALT or AST increased	[4]**Grade 2**: (for pts with normal baseline value ≤ 3.0 x ULN) Maintain the dose level and repeat LFTs as soon as possible, preferably within 48-72 hours from abnormal result awareness. If abnormal lab values are confirmed, decrease dose to the next lower level until resolved to ≤ 3.0 x ULN. Continue to monitor LFTs at least weekly until resolved to ≤ 3.0 x ULN. If resolved ≤ 14 days, then resume initial dose level. If resolved > 14 days, then continue at the decreased dose level.
	[4]**Grade 2**: (for pts with baseline value > 3.0 – 5.0 x ULN): Maintain dose level. Monitor LFTs at least weekly until resolved to ≤ baseline.
	Grade 3: (>5.0 - 10.0 x ULN): Hold dose. Repeat LFTs as soon as possible, preferably within 48-72 hours from abnormal result awareness. Monitor LFTs at least weekly until resolved to ≤ 5.0 x ULN. If resolved ≤ 14 days, then resume same dose level. If resolved > 14 days, then continue at the next lower dose level.
	Grade 3: (>10.0 – 20.0 x ULN): Hold dose. Repeat LFTs as soon as possible, preferably within 48-72 hours from abnormal result awareness. Monitor LFTs at least weekly until resolved to ≤ 5.0 x ULN then resume at the next lower dose level.
	Grade 4: (for pts deriving clinical benefit based on Investigator's judgement): Hold dose. Repeat LFTs as soon as possible, preferably within 48-72 hours from awareness of the abnormal results. Monitor LFTs at least weekly, until resolved to ≤ 3.0 x ULN (or ≤ 5.0 x ULN for participants with baseline value > 3.0 -5.0 x ULN), then resume treatment at next lower dose level. Only 1 dose reduction is allowed; if increase reoccurs at > 5.0 x ULN, drug must be discontinued.
	Grade 4: (for all other pts without clinical benefit): Discontinue ruxolitinib. Repeat LFTs as soon as possible, preferably within 48-72 hours from abnormal result awareness. Monitor LFTs at least weekly until resolved to baseline or stabilized over 4 weeks.
Amylase and/or lipase increased (asymptomatic)	[4]**Grade 3**: Hold dose until resolved to ≤ Grade 2. If resolved in ≤ 7 days, the resume at same dose level. If resolved > 7 days, then resume at next lower dose level.
	[4]**Grade 4**: Discontinue ruxolitinib.
Pancreatitis	**Grade ≥ 3**: Discontinue ruxolitinib.
Diarrhea[3]	[4]**Grade 3**: Decrease to the next lower dose level until resolved to ≤ Grade 2, then resume initial dose level.
	Grade 4: Discontinue ruxolitinib.
Nausea and/or vomiting	[4]**Grade 3**: Hold dose for ≥ Grade 3 vomiting or ≥ Grade 4 nausea only if the vomiting or nausea cannot be controlled with optimal antiemetic (as per local practice)
Hypertension	[4]**Grade 3**: Decrease to the next lower dose level until resolved to ≤ Grade 2, then resume initial dose level.
	Grade 4: Discontinue ruxolitinib.
Rash/photosensitivity	[4]**Grade 3**: Decrease to next lower dose level until resolved to ≤ Grade 2. If resolved in ≤ 7 days, resume initial dose level. If resolved > 7 days, then maintain at the lower dose level.
	Grade 4: Discontinue ruxolitinib.
Other adverse event, determined to be related to ruxolitinib	[4]**Grade** 3: Decrease to next lower dose level until resolved to ≤ Grade 2.
	[4]**Grade 4**: Discontinue ruxolitinib.

[1]Please consult NCI CTCAE Version 5 for complete **Grade** descriptions and note that some grades in Table 2-1 have been split based on action recommended or required.

[2]All actions are mandatory, aside from those identified by an additional footnote. Dose levels are included in Table 2-2.

[3]Antidiarrheal medication is recommended at the first sign of abdominal cramping, loose stools or overt diarrhea.

[4]Actions noted are recommendations only and not mandatory.

Table 2-2: Dose Reduction for ruxolitinib

Current Dose	First Dose Reduction	Second Dose Reduction
10 mg twice daily	5 mg twice daily	5 mg once daily

Participants who have had a dose reduction of ruxolitinib in order to manage toxicity may resume treatment at the previous dose as noted in Table 2-2 if hematologic/non-hematologic parameters meet the required threshold(s) at patient eligibility. Dose re-escalation levels of ruxolitinib are described in Table 2-3. Dose increases may not exceed 10 mg BID, with increments of 5mg twice daily and not more often than every 2 weeks.

Table 2-3: Dose Re-escalation for ruxolitinib

Current Dose	First Dose Escalation	Second Dose Escalation
5 mg once daily	5 mg twice daily	10 mg twice daily
5 mg twice daily	10 mg twice daily	-

2.5.2.6 Ruxolitinib Discontinuation

Ruxolitinib must be discontinued upon any one of the following AE attributed to ruxolitinib that fails to resolve to a Grade 2 or better within 14 days of holding drug, or if a lower re-start dose or administration schedule subsequent to any of the following non-hematologic toxicities is either not available or likely to be clinically ineffective:

- Occurrence of a Grade 4 laboratory or non-laboratory abnormality attributable to ruxolitinib
- Occurrence of a Grade 3 laboratory or non-laboratory abnormality attributable to ruxolitinib that remains at Grade 3 or worse for greater than 14 days
- Withdrawal of consent by the participant
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the opinion of the investigator

2.5.2.7 Corticosteroid Dosing

Participants must be on 2 mg/kg/day of methylprednisolone (or corticosteroid equivalent) at enrollment and continue through the start of ruxolitinib or T-Guard therapy. For participants 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 a 10% dose reduction every 5 days in participants demonstrating CR/PR as observed by the Investigator, beginning no earlier than Day 7 and continuing to approximately Day 56 to allow 7-8 week taper. Each dosing change of corticosteroids must be reported.

2.5.2.8 Calcineurin Inhibitor (CNI) Tapering

Tapering of immunosuppression in responding patients will follow 2 steps:

1. Taper of corticosteroids (see above),
2. Followed by taper of calcineurin inhibitor (CNI)

CNI (cyclosporine or tacrolimus) tapering will follow a 25% dose reduction per month starting from Day 56 in patients demonstrating complete resolution of all signs/symptoms of aGvHD, once off systemic corticosteroids.

2.5.3 Supportive Care

2.5.3.1 GVHD Prophylaxis Medications

Medications such as cyclosporine, tacrolimus, methotrexate (MTX), mycophenolate mofetil (MMF) 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. Close monitoring of drug levels is strongly recommended. Resumption of a GVHD prophylaxis agent is not considered the addition of a second agent.

Overall start and stop dates of immunosuppressants will be captured for this study. However, for agents where dosing is tapered in response to drug levels, the medication dosing will not require reporting.

2.5.3.2 Topical and Ancillary GVHD Therapies

Topical treatment for aGVHD 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 aGVHD 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 transplant-related hepatic toxicity is also allowed according to institutional guidelines.

2.5.3.3 Other Supportive Care Guidelines

In addition to study treatment, all participants 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 participants who develop a positive assay). CMV testing is required weekly through at least Day +56 post randomization. Prophylaxis against CMV is allowed. Any CMV disease requiring treatment will be captured on an Infection Form.

- 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 participants at high risk in accordance with institutional practice. Participants with rapidly rising EBV DNA levels or clinical symptoms are recommended to have imaging studies to diagnose an EBV PTLD. EBV PTLD may rapidly progress and can be fatal if not treated. Management of suspected EBV PTLD should be discussed with one of the Protocol Chairpersons. EBV PTLD can be treated with rituximab and/or infusion of 106 T-cells/kg from the donor. It is recommended that participants with EBV DNA levels of > 1000 copies/mL receive 375 mg/m2 of rituximab. Those participants that continue to have levels above 1000 copies/mL on subsequent testing should be considered to receive three additional weekly infusions of 375 mg/m2 of rituximab. An accelerated schedule of days 1, 4, 8, 15 can be used if there is a suspicion of EBV PTLD. Rituximab has been shown to induce regression in 50 - 70% of cases. Note: Rituximab does not enter the CNS and is not effective in treating CNS disease. Donor lymphocyte infusions may induce regression in > 90% of cases of EBV PTLD and are effective in CNS disease.
- 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.

2.5.3.4 Salvage Treatment Guidelines

Addition of any new systemic immunosuppressive therapy by the Investigator after the start of T-Guard or ruxolitinib treatment is allowed:

- After 7 days for participants meeting aGvHD criteria for progression or mixed response
- After 14 days for participants meeting aGVHD criteria for no response

In case T-Guard infusions are delayed (e.g., for toxicity reasons) the day 7 and 14 timepoints will shift accordingly.

Requirement for initiation of new systemic immunosuppressive therapy will be considered a treatment failure. In this case, the participant must discontinue study treatment.

2.5.3.5 Prohibited Medications

Sirolimus is prohibited from use from the time of enrollment through completion of study drug.

Treatment with any other investigational product is not allowed while on study treatment. An investigational product is defined as medications without any known FDA or EMA approved indications.

2.5.4 Follow-Up Post Treatment Discontinuation

Participants may withdraw from treatment at any time for any reason. The reason should be documented by the study team. However, participants 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 participants that do not receive any study treatment may be replaced. Participants 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 2002 protocol-specific Manual of Procedures (MOP), and the following:

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

The National Marrow Donor Program (NMDP) single Institutional Review Board (IRB) of Record will oversee this study and conduct the study-specific reviews as required by federal regulations and per the NMDP IRB Standard Operating Procedures (SOPs) for centers in the United States. For centers within Europe, the oversight and conduct of the study-specific reviews will be based on the country-specific regulations.

Site personnel will enter data in the electronic case report form (eCRF) in the electronic data capture system (EDC) as described in the BMT CTN 2002 eCRF Completion Guide. Source documentation should be made available for monitoring visits, audits and regulatory inspections as described in the BMT CTN 2002 protocol-specific MOP.

Participating PIs bear ultimate responsibility for training of site staff as well as the scientific, technical, and administrative aspects of conduct of the protocol, even when certain tasks have been delegated to 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 and ICH GCP. 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.

2.7 STUDY TERMINATION

Regulatory agencies have the right to terminate the study at any time in case of safety concerns or if special circumstances concerning the study drug or the company itself occur, making further treatment of participants impossible. The study sponsor also has the right to terminate the study. In this event, the Investigator(s) and relevant authorities will be informed of the reason for study termination.

CHAPTER 3

3 STUDY ENDPOINTS

3.1 ACUTE GVHD RESPONSE DEFINITIONS

Scoring of aGVHD response on a given day is in comparison to the participant's aGVHD staging on the day of randomization. Organ staging will be assessed using the MAGIC criteria (see 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 participant must still be in CR on that day and have had no intervening additional systemic therapy for treatment of 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 participant 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. Participants 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 is defined as progression of aGVHD symptoms requiring additional systemic therapy (including escalation of corticosteroid dose to or beyond 2mg/kg methylprednisolone (or equivalent)); death; or if the participant 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 participants 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 participants 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 $\geq$ 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, participants 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;
- Hypercalcemia (greater than 11 mg/dL);
- Decrease in hemoglobin 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;
- Hyper viscosity related to serum paraprotein.

For the purposes of assessing relapse free survival (RFS), if the participant 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 hypercalcemia 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 treatment of relapse with donor lymphocyte infusion, will be considered evidence of relapse/progression regardless of whether the criteria described above were met. The use of preemptive donor lymphocyte infusion is not considered as relapse.**

***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 participants with a CR on Day 28 after randomization.

3.4 SECONDARY ENDPOINTS

3.4.1 Key Secondary Endpoints:

3.4.1.1 Overall Survival (OS)

OS will be assessed at Days 60, 90 and 180 post-randomization. An event for this analysis is death from any cause and time will be calculated from randomization until date of death.

3.4.1.2 Duration of Complete Response (DoCR)

DoCR is defined as the time from Day 28 until an aGVHD target organ worsens by at least 1 stage and requires a significant escalation in treatment (defined below), or death. The transient worsening of symptoms that resolve without significant escalation of treatment is not considered a loss of CR. A significant escalation in treatment is defined as initiation of new systemic treatment for GVHD and/or escalation in methylprednisolone dose (or equivalent). Methylprednisolone dose increases must be greater than 25% of the current dose and increase at least 8 mg/day (or other steroid equivalent) to be considered an escalation of methylprednisolone. DoCR will be evaluated in the set of participants who are in CR on Day 28 after randomization.

3.4.2 Other Secondary Endpoints:

3.4.2.1 Time to Complete Response

The time from randomization to first observation of CR will be evaluated.

3.4.2.2 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-randomization.

3.4.2.3 Proportion of Response

The proportion of participants in each aGVHD response category will be described at Days 6, 14, 28 and 56 post-randomization.

3.4.2.4 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 randomization until the earlier of death from a non-relapse cause or relapse (competing risk). NRM will be estimated at Days 90 and 180 post-randomization.

3.4.2.5 Relapse-free Survival (RFS)

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

3.4.2.6 GVHD-free Survival

Participants 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-randomization.

3.4.2.7 Chronic GVHD (cGVHD)

cGVHD is defined per NIH Consensus Criteria (see APPENDIX D). Time will be calculated from randomization 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-randomization will be estimated and maximum severity (mild/moderate/severe) will be described.

3.4.2.8 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-randomization will be described.

3.4.2.9 Incidence of Infections

All Grade 2-3 infections (as defined by Appendix G) from randomization will be reported by site of disease, date of onset, and severity. Grade 1 CMV infections requiring treatment that occur post-randomization will also be reported. Incidence of infections will be described in participants

from randomization to 90 days post-randomization. The cumulative incidence of treated CMV post-randomization will be described.

3.4.2.10 Incidence of Toxicities

All Grade 3-5 toxicities according to CTCAE v5 occurring from randomization to Day 56 post-randomization will be described.

3.4.2.11 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:

- C_{inf}: 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.

3.4.2.12 Immunogenicity of T-Guard

ADA responses in the form of human anti-SPV-T3a-RTA and anti-WT1-RTA antibodies will be evaluated with validated bioluminescence assays in serum samples obtained at baseline and at Days 6, 14, 28, 90, and 180 after initiation of treatment in T-Guard treated participants only.

3.5 EXPLORATORY ENDPOINTS

3.5.1.1 Discontinuation of Systemic Steroids

The proportion of participants that is free of systemic steroid therapy at Day 180 post-randomization will be described.

3.5.1.2 Incidence of CMV Reactivation

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

3.5.1.3 Incidence of EBV-associated Lymphoproliferative Disorder

The proportions of participants with EBV-associated lymphoproliferative disorder and EBV reactivation requiring therapy with rituximab by Day 180 post-randomization will be described.

3.5.1.4 Evolution of Cell Populations

The evolution and characterization of specific cell populations over the whole180 day follow-up period will be evaluated in both treatment arms in selected centers. Samples of approximately 50 participants (25 T-Guard, 25 ruxolitinib) will be taken at Day 0, 14, 28, 56 and 180 and either collected in CytoChex preservation tubes, or stored as viably frozen PBMCs, to allow for phenotypic and functional analysis of specific cell subsets, including e.g., FCM and V-beta repertoire analysis. FCM analysis will included the measurement of 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.

3.5.1.5 GVHD-related Biomarkers

GVHD-related biomarker concentrations including serum Interleukin 1 receptor-like 1 (ST2) and Regenerating Family Member 3 Alpha (REG3α) concentrations and urine 3-Indoxyl Sulfate (3-IS) concentrations at baseline and at Day 6, 14, and 28 post-randomization will be used to estimate the probability of NRM at Day 180 post-assessment for each participant, using the NRM risk model from (Major-Monfried, Renteria et al. 2018). The proportion of participants with high-risk biomarker status (defined as estimated NRM greater than 0.29) will be described at each time point.

3.5.1.6 Patient Reported Outcomes (PROs)

Patient reported outcomes will be assessed using a subset of the PROMIS measures described in APPENDIX E. PROs will be assessed at baseline and Days 28, 90, and 180 post-randomization.

3.5.1.7 Incidence of TMA

Incidence of TMA as defined in the section 2.4.2 will be assessed at Day 6, 14, 21, and 28 post-randomization. A blinded review panel will used to review any participants that develop TMA criteria after randomization and treatment.

3.5.1.8 EASIX Score

EASIX score at time of screening will be described.

3.6 ENDPOINT REVIEW PROCESS

Upon completion of participant 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 independent members from centers that are not participating on the study in order to remain unbiased. Each participant's data will be reviewed by ERC clinicians. The adjudicated Day 28 aGVHD response data for each participant will be determined by consensus of the reviewers.

Data will be obtained from the relevant eCRFs and source documents and will be provided to reviewers in a blinded manner with respect to treatment assignment, treatment center, and participant 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 Grade III or IV SR-aGVHD will be approached as soon as possible after diagnosis. The investigator or designee at each study site will evaluate participant eligibility. Informed consent will be obtained via a signature on the IRB or Institutional Ethics Committee (IEC) approved ICF prior to performing study specific procedures. 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

Once the participant has provided a signed consent and eligibility is confirmed, patients will be enrolled and randomized onto the study using the EDC system and assigned a participant identification number. Participants will be randomized to T-Guard versus ruxolitinib in a 1:1 ratio. 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 participant ID number and random treatment assignment is generated upon successful completion of the enrollment form.
3. The confirmation of randomization will be displayed for printing.

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 randomization, ideally within 24 hours. A maximum of 72 hours (3 days) after randomization 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 randomization (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 (Days Post-Randomization)
1 week	6 days[1]
2 weeks	14 days[2]
3 weeks	21 days[2]
4 weeks	28 days[≠]
5 weeks	35 days[2]
6 weeks	42 days[2]
7 weeks	49 days[2]
8 weeks	56 days[2]
10 weeks	70 days[3]
90 days	90 days[3]
6 months	180 days[4]

[1]Target day window for Ruxolitinib arm only is +/-3 days (see section 2.5.1.5 for guidance on T-Guard infusion delays)

[2]Target day window is +/- 3 days

[3]Target window is +/-14 days

[4]Target window is +/-28 days

[≠]Target day window is +/-2 days

Data Collection: The investigator or site designee will enter data collected using an electronic data capture system. In the interest of collecting data in the most efficient manner, the investigator or site designee should transcribe data (including laboratory values, if applicable) in the eCRF in a timely manner after the participant visit unless otherwise noted. The BMT CTN DCC will be reviewing form submission regularly and will follow-up to assure data is submitted promptly.

The investigator or site designee is responsible for ensuring that all data in the eCRFs and queries are accurate and complete and that all entries are verifiable with source documents. These documents should be appropriately maintained by the site.

The monitor will verify a subset of the data in the eCRFs with source documents and confirm that there are no inconsistencies between them.

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 the EDC system within the specified time will be considered delinquent. Transplant Centers can view past due forms via the EDC system. A missing form will continue to appear until the form is entered into the EDC system, 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 Participant Deaths: The Recipient Death Information must be entered into the EDC system within 24 hours of knowledge of a participant'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.

Study Documentation: Study documentation includes, but is not limited to, all eCRFs, workbooks, source documents, monitoring logs and appointment schedules, sponsor-investigator correspondence, and signed protocol and amendments, IRB/IEC correspondence and approved current and previous consent forms and signed participant consent forms.

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study. The original recording of an observation should be retained as the source document. If the original recording of an observation is the electronic record, that will be considered the source.

Clinical Study Monitoring: The Sponsors or delegated Clinical Research Organizations (CROs) are responsible for monitoring the clinical study to ensure that the participant's human rights, safety and well-being are protected, that the study is properly conducted in adherence with the current protocol and GCP and study data reported by the investigator/sub-investigator are accurate and complete, and that they are verifiable with study-related records such as source documents. The Sponsors or delegated CROs are responsible for assigning study monitor(s) to this study for proper monitoring. The protocol will be monitored in accordance with planned monitoring procedures.

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 participants enrolled on this trial. Long-term follow-up of US participants on this study will continue through routine CIBMTR mechanisms. Centers within Europe participating in BMT CTN 2002 should report additional data, including long-term follow-up, per European requirements.

4.3.2 Assessments

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

Prior to Enrollment

The following pre-enrollment assessments must be completed prior to enrolling the participant in the EDC system and within the designated timeframe listed below.

1. Complete aGVHD staging and grading information including assessments of rash, diarrhea, nausea/vomiting, and bilirubin, within 24 - 72 hours prior to enrollment. Biopsy results of involved tissue, if performed as SoC, should also be reported.
2. Medical history within 24 – 72 hours prior to enrollment.

3. Albumin and CK level within 24 – 72 hours prior to enrollment.
4. Complete blood count (CBC) with differential and platelet count within 24 - 72 hours prior to enrollment.
5. Renal function (blood urea and/or blood urea nitrogen, creatinine) within 24 - 72 hours prior to enrollment.
6. Schistocytes, haptoglobin and LDH within 24 - 72 hours prior to enrollment.
7. Pregnancy test (urine or serum) for females of child-bearing potential within 30 days prior to enrollment.
8. Recording of Concomitant medications starting at 7 days prior to randomization

Baseline Assessments

Baseline assessments listed below must be completed at time of enrollment and prior to the first dose of study therapy unless otherwise specified.

1. Physical exam including height and weight, participant disease/transplantation baseline variables
2. Karnofsky Performance Status (may be completed pre- or post-enrollment)
3. Baseline patient-reported outcome measures (see APPENDIX E)
4. Peripheral blood for EBV and CMV viral load (within 72 hours prior to first dose of study drug)
5. CBC with differential and platelet count
6. Recording and review of Concomitant medications
    a. For all immunosuppressant agents, the overall start and stop date will be captured. For agents where dosing is tapered in response to drug levels, the medication dosing will not require reporting.
    b. Dosing for corticosteroids will be captured for all dosing changes.
7. Peripheral blood* for Peripheral Blood Mononuclear Cells (PBMC) testing (See APPENDIX B)
8. Peripheral blood* for Flow Cytometry and Immune Profiling (See APPENDIX B)
9. Peripheral blood* for humoral response (ADA) testing for T-Guard arm only(See APPENDIX B)
10. Peripheral blood* and urine sample* for GVHD biomarker testing (See APPENDIX B)

T-Guard Treatment Assessments

The assessments listed below are to be completed on days of treatment for participants randomized to receive T-Guard. Assessments should be done prior to infusion, unless otherwise specified.

1. Complete aGVHD staging and grading information including assessments of rash, diarrhea, nausea/vomiting, and bilirubin on Days of infusion
2. Vital signs (temperature, pulse, respiratory rate, blood pressure) on Days of infusion . Vital signs should be taken just before starting the infusion, 15 and 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 on Days of infusion
4. CBC with differential and platelet count on Days of infusion

5. Renal function (blood urea and/or blood urea nitrogen, creatinine) on Day of 4th infusion
6. Schistocytes, haptoglobin and LDH on Day of 4th infusion
7. Peripheral blood for EBV and CMV viral load on Day of 4th infusion
8. Recording and review of Concomitant medications on Days of infusions
    a. For all immunosuppressant agents, the overall start and stop date will be captured. For agents where dosing is tapered in response to drug levels, the medication dosing will not require reporting.
    b. Dosing for corticosteroids will be captured for all dosing changes.
9. Recording of AE/SAEs as described in Section 4.4 Adverse Event Reporting
10. Recording of infections
11. Peripheral blood* for humoral response (ADA) on Day of 4th infusion (See APPENDIX B)
12. Peripheral blood* for PK at the following timepoints (See APPENDIX B):
    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 and 24 hours after start of infusion
    c. 3rd infusion: pre-infusion and at 4 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 is allowed for samples drawn at 4 hours after the start of infusion, a window of +/-30 minutes for the sample drawn 5, 6, or 8 hours after the start of infusion, and a window of +/- 1 hour for samples drawn 24 or 48 hours after the start of infusion. Every effort should be made to collect PK samples at all timepoints; however, *no* PK sampling is required on weekend days. Please refer to Section 2.5.1.5 for more information regarding T-Guard infusion delays.

Post-Randomization Assessments

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

1. Complete aGVHD staging and grading information including assessments of rash, diarrhea, nausea/vomiting, and bilirubin at Days 0, 6, 14, 21, 28, 35, 42, 49, 56, 70, 90, and 180
2. cGVHD evaluation (if present) Day 28, 56, 90, and 180
3. Blood pressure at Days 0, 6, 14, 21, 28, 35, 42, 49, 56, 70, 90, and 180
4. Albumin at Days 0, 6, 28, 56, 90, and 180
5. CBC with differential and platelets at Days 0, 6, 14, 21, 28, 56, 90, and 180
6. Renal function (blood urea and/or blood urea nitrogen, creatinine) on Days 6, 14, 21 and 28
7. Schistocytes, haptoglobin and LDH on Days 6, 14, 21 and 28
8. Karnofsky performance status at Days 28, 56, 90, and 180
9. Peripheral blood for EBV and CMV viral load at Days 6, 14, 21, 28, 35, 42, 49, 56 and 180
10. Recording and review of Concomitant medications other than immunosuppressant agents will be collected starting at 7 days prior to randomization through 30 days post-last treatment dose for T-Guard and through Day 44 for ruxolitinib. 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 6,

14, 21, 28, 35, 42, 49, 56, 70, 90, and 180

a. For all immunosuppressant agents, the overall start and stop date will be captured. For agents where dosing is tapered in response to drug levels, the medication dosing will not require reporting.
b. Dosing for corticosteroids will be captured for all dosing changes.

11. Patient reported outcomes at Days 28, 90 and 180 (see APPENDIX E)
12. Recording of toxicities on Days 56, 70, 90, and 180
13. Recording of AE/SAEs as described in Section 4.4 Adverse Event Reporting
14. Recording of infections through Day 180
15. Recording of primary disease relapse through Day 180
16. Peripheral blood* for Peripheral Blood Mononuclear Cells (PBMC) testing at Days 14, 28, 56, and 180 (See APPENDIX B)
17. Peripheral blood* for ADA testing for T-Guard arm only at Days 14, 28, 90, and 180
18. Peripheral blood* for Flow Cytometry and Immune Profiling at Days 14, 28, 56, and 180 (See APPENDIX B)
19. Peripheral blood* and urine sample* for GVHD biomarker testing at Days 6, 14, and 28 (See APPENDIX B)

Please refer to APPENDIX K at the end of the protocol for the Schedule of Assessments tables.

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 participant 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 drug.
- 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 participant and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Medical and scientific judgment should be exercised in deciding whether expected reporting is also appropriate in situations other than those listed above. For example, important medical events may not be immediately life threatening or result in death or hospitalization but may jeopardize the participant or may require intervention to prevent one of the outcomes listed in the definition above (e.g., suspected transmission of an infectious agent by a medicinal product is considered 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. 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.

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 aGVHD 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 aGVHD.

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 reportable 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 de-challenge or re-challenge 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.

4.4.4 Required Adverse Event Reporting

The required adverse event reporting for the BMT CTN 2002 protocol is outlined below.

- T-Guard arm: all adverse events, including SAEs, must be reported from randomization through 30 days following the last dose of T-Guard.
- Ruxolitinib arm: all adverse events, including SAEs, must be reported from randomization through Day 44 (to algin with the maximum reporting period for participants in the T-Guard arm).
- Any SAEs occurring after that period, but assessed as related to the investigational product, must be reported.
- Any unanticipated SAEs from time of enrollment through the study defined follow-up are required to be reported..
- Any grade 4 anticipated event not collected on the calendar-driven toxicity or specified event-driven form must **also** be reported.
- Special Situations and Adverse Events of Special Interest (AESIs) must be reported from randomization through the study defined follow-up period.

- Grade 1 CMV infections, as defined in Appendix G, requiring treatment should be reported from randomization through the study defined follow-up period.

SAEs that require reporting will be reported through an expedited AE reporting system via the EDC system**. All SAEs, Special Situations, and AESIs 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.

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, regardless of grade or seriousness, following the reporting process for SAEs.

The Sponsor has a list of AESIs to be reported. These events include any grade of 1) TMA, 2) CRS, 3) CLS, 4) Myalgia, or 5) CK elevation and 6) grade 3 or higher cytopenias. These events must be reported, regardless of grade or seriousness, following the reporting process for SAEs.

Infections of Grade 2 or 3 by Appendix G 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. Grade 1 CMV infections requiring treatment that occur post-randomization will also be reported using the SAE reporting process.

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.

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, and Death).

The Data and Safety Monitoring Board (DSMB) will receive expedited 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 an annual basis.

4.4.5 Procedure in Case of Pregnancy

If a female participant 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 the EDC system. 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 participant 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.

CHAPTER 5

5 STATISTICAL CONSIDERATIONS

5.1 STUDY DESIGN AND OBJECTIVE

This trial is a randomized, open-label, multi-center Phase 3 study designed to compare the efficacy and safety of T-Guard to ruxolitinib treatment of participants with Grade III-IV SR-aGVHD. A total of 246 participants will be enrolled from approximately 75 transplant centers in the US and Europe. All participants will be followed at minimum through Day 180 post-randomization.

The study will begin with a safety run-in phase of 24 randomized participants (12 to each arm), after which the remaining participants will be enrolled provided that safety concerns do not arise during the run-in.

5.1.1 Accrual and Study Duration

Accrual is estimated to require 34 months (9-10 participants enrolled per month at full enrollment) to complete with a total study duration of 40 months.

5.1.2 Randomization and Blinding

Participants will be randomized at the time of enrollment in a 1:1 ratio to the treatment arms. Randomization will be stratified by center region (US vs. Europe) and age group (at least 55 years vs. under 55). Stratified treatment assignments will be generated using permuted blocks of random sizes.

As this is an open-label trial, no blinding will be performed.

5.1.3 Analysis Populations

The primary analysis population will include all randomized participants classified according to the intention-to-treat (ITT) principle, that is, according to their randomized assignment.

A secondary, supportive analysis of a per protocol (PP) population will be performed. The PP population will consist of all participants from the ITT population who received at least one dose of the assigned treatment.

A PK analysis population will be defined for the assessment of pharmacokinetic and immunogenicity endpoints in the T-Guard arm only. This will consist of the participants randomized to T-Guard from whom samples have been collected and evaluated for these endpoints.

Note that because the safety run-in phase is randomized, the inclusion of its participants in the analysis will not contribute bias. Therefore, run-in participants will be included in the ITT population and in the PP and PK populations as long as the other requirements for inclusion are satisfied.

5.1.4 Primary Hypothesis

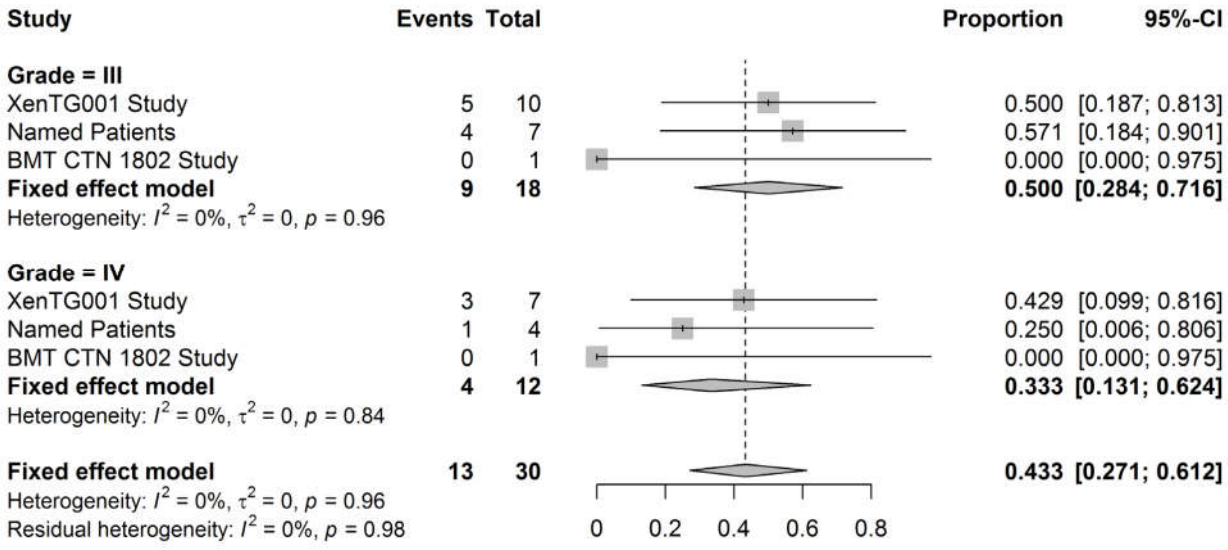
The primary endpoint is the attainment of a CR at Day 28 post-randomization. This requires three conditions to be satisfied: (i) a score of 0 for aGVHD staging in target organs at Day 28; (ii) the participant is still alive at Day 28; and (iii) no additional systemic treatment for aGVHD has been administered through Day 28.

The primary hypothesis is that the proportion of participants with a CR at Day 28, called the Day 28 CR rate, is greater for the T-Guard arm compared to the ruxolitinib arm. This hypothesis will be evaluated by comparing the null hypothesis that the Day 28 CR rate under T-Guard treatment is no better than this rate under ruxolitinib treatment to the alternative hypothesis that this rate is superior for the T-Guard arm. This evaluation will be performed by a one-sided test at a 2.5% significance level, adjusted for the factors used to stratify the randomization (region and age) and for acute GVHD grade at randomization (III vs. IV).

5.1.5 Sample Size and Power Considerations

Data on participants with Grade III-IV SR-aGVHD were examined from three previous T-Guard studies in a meta-analysis: the Phase 1/2 study (XenTG001), an Expanded Access Program for this study (Named Participants), and the Phase 3 study BMT CTN 1802. Estimates of Day 28 CR rates for each cohort and for the combined participant populations are displayed in Figure 5-1. Aggregating data from these 30 participants gives an estimated CR rate under T-Guard treatment of 43.3% (95% CI: 27.1% - 61.2%). Among the 101 Grade III-IV SR-aGVHD participants in the ruxolitinib arm of the REACH2 trial, the CR rate was 25.7% (95% CI: 18.2% - 35.1%). These data suggest Day 28 CR rates of approximately 43% and 26% for T-Guard and ruxolitinib, respectively.

Figure 5-1: Day 28 CR Rates from Meta-analysis of Previous T-Guard Trials



The primary hypothesis will be evaluated by comparing the proportion of participants attaining a Day 28 CR in each arm, aiming to detect an improvement for T-Guard treatment over ruxolitinib. If the true CR rates for T-Guard and ruxolitinib match the respective rates of 43% and 26% seen in previous studies, the difference in rates is 17% favoring T-Guard. With the inclusion of interim analyses for efficacy and futility as detailed in Section 5.2, 246 participants are required to detect this difference with 80% power and a one-sided type I error rate of 2.5%. The pivotal analysis will also adjust for the stratification factors used for randomization. This practice is known to increase statistical power beyond that of an unadjusted two-arm comparison (Kahan and Morris 2012), so 80% is a conservative estimate of the true power.

5.2 STUDY ANALYSIS SCHEDULE

Figure 5-2 illustrates the study design and the analyses to be conducted during the course of the trial.

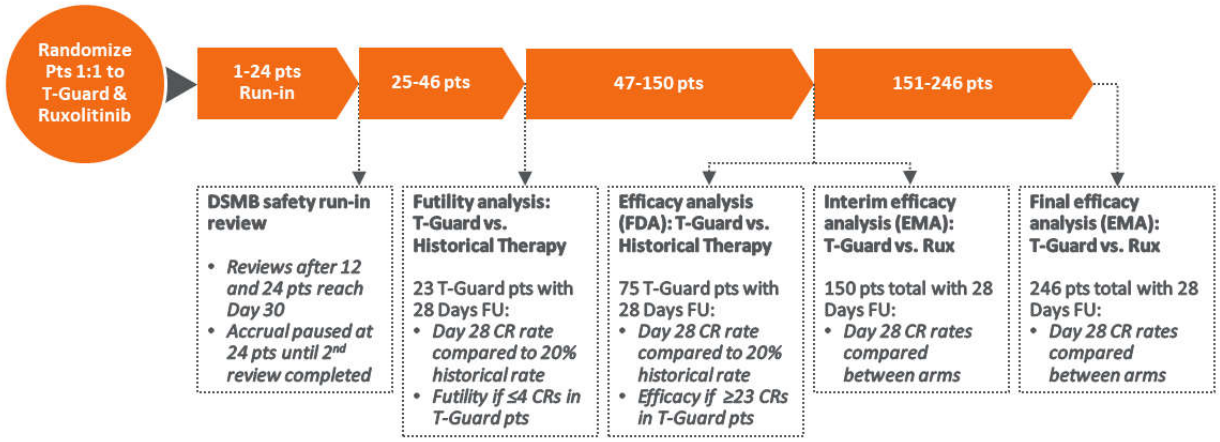
After accrual of 12 T-Guard participants (~24 participants in combined arms), the study enrollment will be paused to permit the DSMB to perform a comprehensive review of safety data and confirm that the study may continue (see section 5.3.1). Efficacy of T-Guard will not be formally evaluated during this safety review. Upon DSMB approval, the study will continue for full enrollment.

Once 23 T-Guard participants (~46 participants in combined arms) complete follow-up through Day 28, a futility analysis (see section 5.2.1.1) will compare the CR rate in T-Guard participants to a historical therapy CR rate of 20%.

After 150 participants reach Day 28, two separate analyses will be performed to test whether T-Guard's Day 28 CR rate is superior to:

1. Historical therapy (see section 5.2.1.2), to support T-Guard approval from US regulatory authorities (FDA).
2. Ruxolitinib treatment (see section 5.2.2), to support T-Guard approval from European regulatory authorities (EMA). The superiority of the Day 28 CR rate for T-Guard treatment compared to ruxolitinib treatment will be evaluated. The analysis will take place when:
   a. 150 participants have reached their day 28 assessment and 100 participants have at least 6 months follow-up.
   b. If efficacy is not found at this analysis, an analysis will follow once all 246 participants have been enrolled and reached their day 28 assessment (see section 5.2.2). This analysis serves as the final analysis of the primary endpoint. The final analysis of other endpoints will be conducted once all 246 participants complete the 180-day follow-up period.

Figure 5-2: BMT CTN 2002 Study Design and Interim Analysis Plan



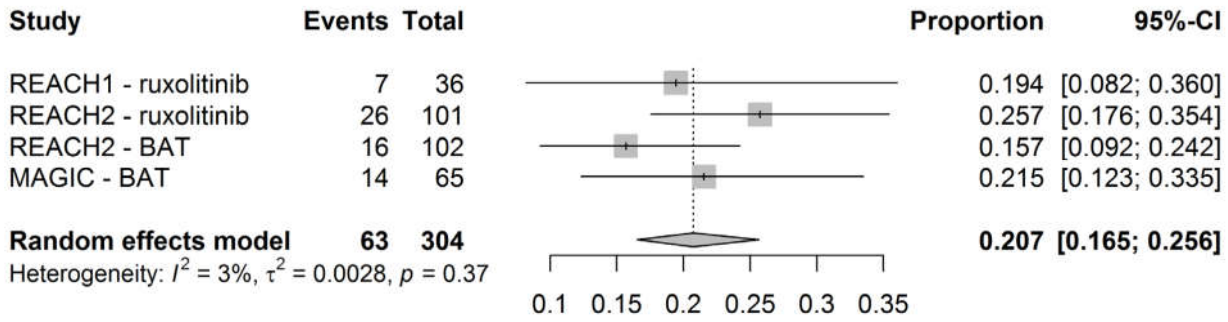
5.2.1 Simon's Two-Stage Design for Demonstrating Superiority of T-Guard to Historical Therapies for Grade III-IV SR-aGVHD

Single arm evaluations of the T-Guard CR rate alone will be performed to demonstrate improvement over a clinically relevant Day 28 CR rate for patients with Grade III-IV SR-aGVHD, as requested by the FDA. This CR rate will serve as the null hypothesis for the Simon's two-stage analysis. This clinically relevant response rate was determined taking the following into account:

- A meta analysis was performed of the Day 28 CR rates observed in patients with Grade III-IV SR-aGVHD in the REACH1 (ruxolitinib) and REACH2 (ruxolitinib and BAT) studies and in Grade III-IV SR-aGVHD patients from the MAGIC database (see Figure 5-3) who received second line therapy. This analysis included 304 patients in total and observed a Day 28 CR rate of 20.7% in the combined cohort.
- FDA's approval of ruxolitinib for the treatment of SR-aGVHD is based on the Day 28 ORR rate observed in the 47 patient efficacy cohort of the REACH1 registration study of patients with Grade II-IV SR-aGVHD. Among patients in this efficacy cohort with Grade III-IV SR-aGVHD, ruxolitinib induced a Day 28 CR rate of 19.4% (95% CI: 8.2 - 36.0%).

Based on the above, the study team considers a Day 28 CR rate of 20% to be clinically relevant. The two-stage design is organized such that if efficacy of T-Guard is found, the entire 95% CI for the T-Guard Day 28 CR rate will lie above this 20% rate. In particular, it will exclude the 19.4% CR rate observed in Grade III-IV SR-aGVHD patients treated by ruxolitinib in the efficacy cohort of REACH1.

Figure 5-3: Day 28 CR Rates from Meta-analysis of Previous Ruxolitinib and Best Available Therapy Studies



Per FDA requirement, the primary endpoint for the FDA analysis is the attainment of a CR at Day 28 post-T-Guard initiation. The FDA analysis population will include patients who are randomized to T-Guard and initiate this treatment. This approach is consistent with the analysis employed in the single arm REACH1 trial. A secondary analysis of this endpoint will add to the FDA analysis population patients who were randomized to T-Guard but failed to initiate treatment, classifying these patients as non-responders.

The superiority of this T-Guard CR rate to a historical, clinically relevant therapy rate of 20% will be tested using a Simon's two-stage design at a type I error rate of 2.5%. The design is summarized in Table 5-1. Stage 1 will evaluate 23 T-Guard participants and declare futility if 4 or fewer CRs are observed. Stage 2 will evaluate 75 T-Guard participants and declare efficacy in contrast to historical therapies if 23 or more CRs are seen. This testing procedure provides 98% power to detect a 23% improvement in the T-Guard CR rate over the 20% historical therapy rate. The use of these stages for decision making is described in subsequent sections.

Table 5-1: Simon's Two-Stage Design for Comparing T-Guard to Historical Therapies

Stage	Number of T-Guard Participants	Futility Boundary – # of CRs (CR rate)	Probability of Declaring Futility Under True T-Guard CR Rate Below		
			20%	26%	43%
1	23	4 (17.4%)	50.1%	24.8%	0.9%
2	75	22 (29.3%)	-	-	-

5.2.1.1 Futility Analysis (Stage 1)

An interim analysis for futility will be performed when 23 participants on the T-Guard arm complete the Day 28 assessment. This futility analysis will be based on the first stage of the two-stage design (see Table 5-1), with futility declared if 4 or fewer CRs are observed out of the 23 participants (observed CR rate of 17.4% or less). This is a non-binding futility analysis in that the overall type I error rate will not be inflated should the futility rule not be followed exactly.

5.2.1.2 Efficacy Analysis Toward FDA Approval (Stage 2)

An analysis of the T-Guard CR rate will be performed toward obtaining FDA approval once 150 participants (75 per arm) complete the Day 28 assessments and 100 participants have completed the Day 180 assessment. This analysis will consider the CR rate specifically in the 75 participants in the T-Guard arm and will compare this to historical therapies using the second stage of the Simon's design (see Table 5-1). If 23 or more CRs are observed in this set (30.7% or higher), this will provide evidence of superiority of T-Guard in comparison to historical therapies and will support a request for FDA regular approval. Point and interval estimates of the T-Guard CR rate will be provided for this analysis using the minimum variance unbiased estimator and stagewise ordering confidence intervals proposed in Koyama et al. 2008. The additional requirement of 100 participants with 180 days of follow-up will ensure that sufficient data are available for a safety analysis that would be provided with the application for FDA approval.

5.2.2 Efficacy Analysis for Demonstrating Superiority of T-Guard to Ruxolitinib Toward EMA Approval

An interim analysis for efficacy will be conducted once 150 participants (75 per arm) complete the Day 28 assessments and 100 participants have completed the Day 180 assessment. This analysis will compare the Day 28 CR rates between trial arms (T-Guard vs. ruxolitinib) using logistic regression while adjusting for the stratification factors. An error spending approach will be employed to control the overall type I error rate using the O'Brien-Fleming spending function. Operating characteristics for this design are shown in Table 5-2. This analysis in 150 participants will correspond to a one-sided significance level of approximately 0.44% as shown in the table. If superiority is not found at this analysis, a final analysis will follow once all 246 participants have completed the Day 28 assessment, at which the remaining type I error rate will be spent. A finding of efficacy for T-Guard at either this interim or final analysis will be used to support an application for EMA approval.

Table 5-2: Operating Characteristics for Trial Design

Efficacy Analysis	Sample Size	Information Fraction	Efficacy Boundary		Cumulative Type I Error Rate	Cumulative Power Under Alternative
			Difference in CR Rates	Z Statistic		
1	150	61.0%	20.19%	2.6562	0.44%	34.14%
2	246	100.0%	11.82%	1.9817	2.37%	80.56%

*Type I error rate / power calculations obtained from 100,000 Monte Carlo simulations while accounting for futility analysis in Section 5.2.1.1

5.3 GUIDELINES FOR SAFETY MONITORING AND RUN-IN PHASE

The occurrence of adverse events, toxicity, and other safety endpoints will be monitored regularly. These data will be reported to the DSMB at annual meetings at a minimum; in the event that any safety concerns arise, these data will be conveyed to the DSMB expeditiously. The policies and composition of the DSMB are described in the BMT CTN 2002 protocol-specific

MOP. In addition, monitoring of key safety endpoints will be conducted daily. If their rates significantly exceed pre-set thresholds, the NHLBI will be notified in order that the DSMB can be advised. These monitoring 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.

5.3.1 Safety Run-in Phase

The first portion of the trial will consist of a run-in phase, comprised of the first 12 patients randomized to the T-Guard arm and concurrently enrolled patients randomized to the ruxolitinib arm (approximately 24 patients total). During this phase:

- A stopping rule will monitor the Day 30 mortality rate in the T-Guard arm (see section 5.3.2).
- While the first 6 T-Guard patients are accrued, enrollment will be paused if this T-Guard arm monitoring rule (see Table 5-3) can possibly be triggered under the current enrollment and will be resumed when an additional T-Guard patient survives past Day 30. Specifically:
    - After 3 T-Guard patients are enrolled, additional enrollment will be permitted when 1 T-Guard patient has survived past Day 30.
    - After 4 T-Guard patients are enrolled, additional enrollment will be permitted when 2 T-Guard patients have survived past Day 30.
    - After 6 T-Guard patients are enrolled, additional enrollment will be permitted when 3 T-Guard patients have survived past Day 30.
- An additional stopping rule will compare the Day 60 mortality rates between the T-Guard and ruxolitinib arms throughout the run-in and post run-in phases of the trial (see section 5.3.2 and Table 5-7.)
- The DSMB will conduct two comprehensive reviews of early safety data: once 6 participants in the T-Guard arm have completed follow-up through Day 30, and when 12 participants in this arm have reached Day 30.

Enrollment will be paused following enrollment of the 12th T-Guard patient in order to permit the DSMB to thoroughly examine these participants’ data and certify that the trial should open to unrestricted enrollment.

5.3.2 Safety Endpoint Monitoring

A key safety endpoint for this study is Day 30 overall mortality post-randomization. Based on historical data from the REACH trials and MAGIC, the expected probability of Day 30 mortality in Grade 3-4 SR-aGVHD participants is 15-20%, while a rate of 30% is considered to be unacceptable. A sequential probability ratio test (SPRT) for binary data will be used for daily monitoring of the Day 30 mortality rate within the T-Guard arm during the run-in phase, comparing a rate of 15% under the null hypothesis to a rate of 30% under the alternative hypothesis.

This sequential testing procedure preserves the type I error rate at a prespecified level across all of the daily examinations. The binary SPRT can be represented graphically, with the continuation region of the SPRT defined by two parallel lines. At each examination, the number

of evaluable participants is plotted against the cumulative number of events. Only the upper boundary will be used for monitoring in order to protect against excessive Day 30 mortality. If the cumulative number of deaths meets or exceeds 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 participants. Otherwise, the SPRT continues until the run-in phase enrollment reaches the target sample size of 12 participants in the T-Guard arm. Nominal respective type I and type II error rates of 15% and 10% each were used to specify the rejection boundary.

Table 5-3 displays the SPRT in a tabular form, showing the rejection boundaries for the number of Day 30 mortality events corresponding to the number of evaluable participants. At least three deaths must be observed in order to trigger review.

Table 5-3: Sequential Monitoring Plan of Day 30 Overall Mortality in T-Guard Arm During Run-in Phase

Number of Evaluable Participants	Rejection Boundary for Number of Day 30 Deaths
3 - 4	3
5 - 9	4
10 - 12	5

The operating characteristics of the truncated test are shown in Table 5-4. They were obtained from a simulation study that assumed uniform accrual of 12 participants to the T-Guard arm during the run-in phase. During the run-in phase, this procedure rejects the null hypothesis in favor of the alternative 4.6% of the time when the true Day 30 mortality rate is 15% and 74.5% of the time when the rate is 45%. If the true Day 30 mortality rate is 45%, the DSMB will, on average, be consulted when 4 events have been observed in 9 T-Guard arm participants. The limited size of the run-in phase permits reliable detection only of a true mortality rate in excess of 40% but provides some assurance of the safety of T-Guard before proceeding with full enrollment.

Table 5-4: Operating Characteristics of the Binary SPRT for Single Arm Day 30 Overall Mortality Monitoring During Run-in Phase

True Day 30 Mortality Rate	15%	20%	25%	30%	35%	40%	45%	50%
Probability of Early Stopping	4.6%	11.4%	21.6%	34.5%	48.6%	62.3%	74.5%	84.1%
Mean # Deaths by Day 30	1.8	2.3	2.8	3.1	3.4	3.6	3.7	3.7
Mean # T-Guard Participants Enrolled	11.8	11.6	11.2	10.8	10.2	9.5	8.8	8.2

*Operating characteristics obtained from 100,000 Monte Carlo simulations

A second monitoring rule will evaluate whether the Day 60 mortality risk post-randomization for the T-Guard arm exceeds that for the ruxolitinib arm throughout the trial. Based on historical

data from the REACH trials and MAGIC, the expected probability of Day 60 mortality in Grade 3-4 SR-aGVHD participants is 40%, while a rate of 65% is considered to be unacceptable. The presence of excess Day 60 mortality risk in the T-Guard arm evaluated using an SPRT that compares a null odds ratio of 1 for Day 60 mortality risk under T-Guard vs. ruxolitinib to an alternative value of 2.79. The alternative odds ratio of 2.79 corresponds to the increase in risk produced if the ruxolitinib rate equals 40% as expected while the T-Guard mortality rate is higher at 65%.This rule will only consider early stopping to reject the null hypothesis of equal mortality risk. An aggressive rejection boundary will be used during the run-in phase in order to provide enhanced vigilance in comparing risk between arms, determined by nominal type I and II error rates of 20.5% and 25%, respectively. Following the run-in, the rejection boundary is determined by nominal type I and II error rates of 5% and 10%.

At each evaluation, this SPRT will use the conditional likelihood of the odds ratio given the total number of deaths observed in both arms, with excess risk for T-Guard indicated if a disproportionately high number of deaths occurred in that arm. As with the binary SPRT for single arm monitoring, this procedure preserves the type I error rate at a prespecified level across all of the daily examinations. Additional details for this monitoring rule are included in APPENDIX F.

The probability of stopping early under the monitoring scheme, that is triggering at least one monitoring rule (single arm and/or comparative), is shown in Table 5-5.under a range of potential Day 60 mortality rates for the T-Guard and ruxolitinib arms. These were obtained from a simulation study that assumed uniform accrual of 123 participants per arm over the entire trial duration. The entries on the main diagonal correspond to situations where the mortality risk is identical between arms; for these, the testing procedure rejects the null hypothesis of equal risk approximately 10 - 12% of the time when the common mortality rate is 40% or less, indicating that the type I error rate lies within this range. For scenarios where the risk under T-Guard is 20% higher than that for ruxolitinib, the SPRT rejects the null hypothesis in approximately 84-87% of cases, demonstrating that this testing procedure offers 84+% power to detect T-Guard mortality rates that are 20+% higher than ruxolitinib.

Table 5-6 displays the average number of deaths for each arm under the monitoring scheme for the range of true mortality rates considered, accounting for any early stopping that may occur as described in Table 5-5.

Table 5-5: Probability of Early Stopping for Day 60 Overall Mortality Signal under Combined Monitoring Scheme During the Entire Trial

Stopping Probability		True T-Guard Mortality Rate								
		30%	35%	40%	45%	50%	55%	60%	65%	70%
True Ruxolitinib Mortality Rate	30%	10.7%	22.2%	41.4%	65.9%	85.6%	96.2%	99.4%	99.9%	100.0%
	35%	5.9%	11.6%	22.2%	40.5%	64.1%	84.9%	95.8%	99.4%	100.0%
	40%	3.7%	6.7%	12.2%	22.4%	40.3%	63.8%	84.6%	95.9%	99.4%
	45%	2.3%	4.3%	7.2%	13.1%	23.0%	40.7%	64.3%	85.3%	96.5%
	50%	1.4%	2.7%	4.7%	8.2%	13.9%	24.6%	42.7%	66.9%	87.6%
	55%	0.9%	1.8%	3.1%	5.4%	9.2%	15.8%	27.4%	46.9%	71.5%
	60%	0.5%	1.1%	2.1%	3.8%	6.7%	11.5%	19.2%	32.5%	53.4%
	65%	0.4%	0.7%	1.5%	2.8%	5.4%	9.5%	15.8%	25.6%	40.9%
	70%	0.2%	0.5%	1.0%	2.3%	4.6%	8.4%	14.5%	23.2%	35.5%

*Operating characteristics obtained from 100,000 Monte Carlo simulations.
Rejection criteria of both the single arm and double arm monitoring rules considered.

Table 5-6: Average Number of Deaths by Day 60 by Arm under Combined Monitoring Scheme During the Entire Trial

# T-Guard Deaths, # Rux Deaths		True T-Guard Mortality Rate								
		30%	35%	40%	45%	50%	55%	60%	65%	70%
True Ruxolitinib Mortality Rate	30%	33.6, 33.6	35.9, 30.7	35.3, 26.5	31.1, 20.7	25.2, 15.1	19.2, 10.5	14.7, 7.4	11.6, 5.4	9.5, 4.1
	35%	35.0, 40.7	38.8, 38.8	40.7, 35.6	39.5, 30.7	34.9, 24.4	27.8, 17.7	21.1, 12.3	15.7, 8.5	12.2, 6.1
	40%	35.7, 47.5	40.4, 46.2	44.0, 44.0	45.6, 40.5	43.7, 35.0	38.2, 27.7	30.1, 20.1	22.3, 13.7	16.3, 9.3
	45%	36.1, 54.2	41.3, 53.2	46.0, 51.7	49.1, 49.0	50.2, 45.1	47.5, 38.9	40.7, 30.5	31.3, 21.7	22.4, 14.4
	50%	36.4, 60.7	41.9, 60.0	47.1, 58.8	51.2, 56.9	54.0, 54.0	54.2, 49.3	50.3, 41.9	41.8, 32.2	30.9, 22.1
	55%	36.6, 67.1	42.3, 66.5	47.8, 65.6	52.6, 64.2	56.3, 61.9	58.2, 58.2	57.1, 52.4	51.3, 43.4	40.8, 32.0
	60%	36.7, 73.4	42.6, 73.1	48.2, 72.4	53.4, 71.2	57.7, 69.2	60.5, 66.0	61.2, 61.2	58.0, 53.5	49.8, 42.6
	65%	36.8, 79.7	42.8, 79.4	48.5, 78.8	53.9, 77.8	58.4, 75.9	61.7, 72.9	63.1, 68.3	61.5, 61.5	55.6, 51.7
	70%	36.8, 85.9	42.9, 85.7	48.7, 85.3	54.2, 84.3	58.8, 82.4	62.4, 79.4	63.9, 74.6	62.9, 67.7	58.3, 58.3

*Operating characteristics obtained from 100,000 Monte Carlo simulations.
Rejection criteria of both the single arm and double arm monitoring rules considered.

The halting criteria are summarized in Table 5-7 for the first 6 participants per arm. Because the halting rule at any given examination depends on the numbers of events and evaluable participants in both arms, the rule cannot be easily tabulated for the entire run-in phase or trial. The table includes the decisions made in situations where the T-Guard arm has 1 or more excess deaths in comparison to the ruxolitinib arm.

Table 5-7: Safety Monitoring Scheme for Day 60 Mortality within First 6 Participants per Arm for Event Scenarios of Special Interest

		T-Guard (# Day 60 Deaths / # Evaluable)											
		2/3	3/3	2/4	3/4	4/4	3/5	4/5	5/5	3/6	4/6	5/6	6/6
Ruxolitinib (# Day 60 Deaths / # Evaluable)	0/3												
	1/3												
	0/4												
	1/4												
	0/5												
	1/5												
	2/5												
	0/6												
	1/6												
	2/6												

* Green = continue; red = pause

5.4 DEMOGRAPHIC AND BASELINE CHARACTERISTICS

Demographic and baseline characteristics will be summarized for all participants and by treatment arm. Characteristics to be examined include age, gender, race/ethnicity, performance status, primary disease, primary disease status, disease risk index (DRI), time from steroid initiation to enrollment, aGVHD grade and organ staging, graft source, GVHD prophylaxis, conditioning regimen, time from disease diagnosis to transplant, time from transplant to enrollment, time from aGVHD onset to enrollment, and EASIX score.

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.

5.5 ANALYSIS OF PRIMARY ENDPOINT

The primary endpoint of this trial is the attainment of a CR at Day 28 post-randomization. The primary hypothesis is that the proportion of participants with a CR at Day 28, called the Day 28 CR rate, is greater for the T-Guard arm compared to the ruxolitinib arm. This hypothesis will be evaluated by comparing the null hypothesis that the Day 28 CR rate under T-Guard treatment is no better than this rate under ruxolitinib treatment to the alternative hypothesis that this rate is superior for the T-Guard arm.

The estimand for Day 28 CR is based on the composite strategy, where a participant is classified as a CR provided three conditions are satisfied:

1. Stage 0 aGVHD in target organs at Day 28
2. The participant is still alive at Day 28
3. No additional systemic treatment for aGVHD has been administered through Day 28

Participants who do not satisfy all three criteria will be classified as failures for CR.

The following intercurrent events could potentially impact the pivotal analysis: discontinuation of study drug before Day 28, and missingness of the Day 28 GVHD assessment. The pivotal analysis accounts for these events as follows:

- Discontinuation of study drug will be carefully monitored. Under the ITT principle, the analysis includes all randomized participants, so treatment discontinuation itself has no effect on the analysis because it produces no changes to the participant population or evaluation of CR criteria. Initiation of additional systemic aGVHD treatment following discontinuation would be considered a failure for CR per the composite strategy described above.
- Participants whose Day 28 GVHD assessment is missing for any reason (including loss to follow-up, withdrawal from study, or a missed visit) will be classified as failures (non-CRs) for the primary endpoint.

The pivotal analysis for this study involves comparison of the Day 28 CR rate between arms in the primary (ITT) analysis population. The summary measure used to compare Day 28 CR rates between T-Guard and Ruxolitinib is the odds ratio, to be evaluated using logistic regression while adjusting for region (US vs. Europe) and age (at least 55 years vs. under 55) and acute GVHD grade at randomization (Grade III vs. IV). Testing will be one-sided at an overall significance level of 0.025. Two efficacy analyses for the primary endpoint will be performed as described in Section 5.2.2, one interim and one final, with the O'Brien-Fleming error spending function used to determine the appropriate critical values for each analysis.

The Day 28 CR rate will be described for each arm using the sample proportions and corresponding 95% confidence intervals.

A secondary analysis will assess the proportions of treated participants who attain a CR at Day 28 post-treatment initiation. This set of participants will include those from the PP population who are not in CR at the time of treatment initiation, with symptoms compared from the time of initiation to 28 days following initiation to determine CR status. This Day 28 CR rate will be described for each arm using the sample proportions and corresponding 95% confidence intervals. Day 28 CR rates will be compared between T-Guard and ruxolitinib using logistic regression while adjusting for region (US vs. Europe) and age (at least 55 years vs. under 55) and acute GVHD grade at randomization (Grade III vs. IV).

5.6 ANALYSIS OF SECONDARY ENDPOINTS

The analysis of secondary endpoints will be based on the primary ITT analysis population. A supplemental analysis will also be performed in the PP population.

Key Secondary Endpoints:

The study has two key secondary endpoints: OS and DoCR. The comparison of OS between treatment arms will be alpha-protected; to protect the familywise type I error rate of 2.5%, a gatekeeping procedure will be employed where OS will be tested for superiority of T-Guard to ruxolitinib only if a significant benefit of T-Guard is found in the pivotal analysis of the primary endpoint.

5.6.1 Overall Survival (OS)

OS is defined as survival of death from any cause. The time from randomization until death from any cause will be described for each arm using the Kaplan-Meier estimator. Estimates and 95% CIs for OS will be provided at Days 60, 90 and 180 post-randomization for each arm.

Provided that the pivotal analysis demonstrates superiority of T-Guard to ruxolitinib, a Cox proportional hazards model will compare the overall mortality hazard rates between the T-Guard and ruxolitinib arms through Day 180 while adjusting for the stratification factors and for acute GVHD grade at randomization (Grade III vs. IV).

5.6.2 Duration of Complete Response (DoCR)

DoCR will be evaluated only in the set of participants who are in CR at Day 28 post-randomization. The primary definition of DoCR is the time from Day 28 until an aGVHD target organ worsens by at least 1 stage and requires a significant escalation in treatment (defined below), or death. The transient worsening of symptoms that resolve without significant escalation of treatment is not considered a loss of CR. A significant escalation in treatment is defined as initiation of new systemic treatment for GVHD and/or escalation in methylprednisolone dose (or equivalent). Methylprednisolone dose increases must be greater than 25% of the current dose and increase at least 8 mg/day (or other steroid equivalent) to be considered an escalation of methylprednisolone. DoCR will be described for each arm using the Kaplan-Meier estimator, with estimates and 95% CIs for the median of DoCR obtained from this estimator using the method described in Andersen et al. 1993, Chapter IV.

A Cox proportional hazards model will compare the hazard rates of loss of CR between the T-Guard and ruxolitinib arms through Day 180 while adjusting for the stratification factors. This will include DoCR events as defined in the previous paragraph.

A secondary analysis will use an alternative definition of DoCR as the time from Day 28 until death or a significant escalation of aGVHD therapy occurs, as defined in the primary analysis of DoCR.DoCR defined in this manner will be described for each arm using the Kaplan-Meier estimator, with estimates and 95% CIs for the median of DoCR obtained from this estimator using the method described in Andersen et al. 1993, Chapter IV.

Other Secondary Endpoints:

5.6.3 Time to Complete Response (CR)

The time from randomization until first attaining a CR will be described for each treatment arm using the Aalen-Johansen estimator, with death and additional systemic treatment for aGVHD treated as competing risks. Estimates and 95% CIs of the cumulative incidence of CR will be

provided at Days 28 and 56 post-randomization. The cause-specific hazards of CR will be compared between arms using a Cox proportional hazards model with adjustment for the stratification factors and for acute GVHD grade at randomization (Grade III vs. IV).

5.6.4 Overall Response Rate (ORR)

Overall response is defined as either a complete or participant response (CR+PR). The ORR will be estimated at Days 14, 28, and 56 post-randomization for each treatment arm using sample proportions and 95% Wilson score or Clopper-Pearson CIs, as appropriate. ORRs will be compared between arms by evaluating the odds ratio of CR/PR for T-Guard vs. ruxolitinib using logistic regression with adjustment for the stratification factors and for acute GVHD grade at randomization (Grade III vs. IV).

5.6.5 Proportion of Response

The proportion of participants in each aGVHD response category will be described at Days 6 14, 28 and 56 post-randomization for each treatment arm. These proportions will be compared between arms using Pearson's chi-square test or Fisher's exact test as appropriate.

5.6.6 Non-relapse Mortality (NRM)

NRM is defined as death from any cause other than malignancy relapse/progression. The time from randomization until NRM will be described for each treatment arm 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-randomization. The cause-specific hazards of NRM will be compared between arms using a Cox proportional hazards model with adjustment for the stratification factors and for acute GVHD grade at randomization (Grade III vs. IV).

5.6.7 Relapse-free Survival (RFS)

RFS is defined as being alive and free of malignancy relapse/progression. The time from randomization until malignancy relapse/progression or death will be described for each arm using the Kaplan-Meier estimator. Estimates and 95% CI for RFS at Day 180 post-randomization will be provided. A Cox proportional hazards model will compare the hazard rates of relapse/progression/mortality between the T-Guard and ruxolitinib arms with adjustment for the stratification factors and for acute GVHD grade at randomization (Grade III vs. IV).

5.6.8 GVHD-free Survival

GVHD-free survival is defined as being alive, in CR, and free of cGVHD. The proportion of participants with GVHD-free survival at Days 90 and 180 post-randomization will be estimated for each treatment arm using sample proportions and 95% Wilson score or Clopper-Pearson CIs, as appropriate. The proportions of participants with GVHD-free survival at Day 180 will be compared between arms using logistic regression with adjustment for the stratification factors and for acute GVHD grade at randomization (Grade III vs. IV).

5.6.9 Chronic GVHD (cGVHD)

cGVHD severity is defined per the 2014 NIH Consensus Criteria (see APPENDIX D). The maximum severity of cGVHD through Day 180 post-randomization will be tabulated by arm. The time from randomization until onset of cGVHD of any severity (mild, moderate, or severe) will be described for each treatment arm using the Aalen-Johansen estimator, with death prior to cGVHD onset treated as a competing risk. Estimates and 95% CIs of the cumulative incidence of cGVHD will be provided at Day 180 post-randomization. The cause-specific hazards of cGVHD will be compared between arms using a Cox proportional hazards model with adjustment for the stratification factors and for acute GVHD grade at randomization (Grade III vs. IV).

5.6.10 Relapse/Progression of Underlying Malignancy

The time from randomization until malignancy relapse/progression will be described for each treatment arm using the Aalen-Johansen estimator, with death prior to relapse/progression treated as a competing risk. Estimates and 95% CIs of the cumulative incidence of malignancy relapse/progression will be provided at Day 180 post-randomization. The cause-specific hazards of relapse/progression will be compared between arms using a Cox proportional hazards model with adjustment for the stratification factors and for acute GVHD grade at randomization (Grade III vs. IV).

5.6.11 Incidence of Infections

The frequency of Grade 2-3 infections occurring from randomization until Day 90 post-randomization will be tabulated by infection site, date of onset, and severity, with Grade defined per Appendix G. Grade 1 CMV infections requiring treatment that occur post-randomization will also be summarized. The cumulative incidence of Grade 2-3 infections at Day 90 post-randomization will be described by treatment arm using the Aalen-Johansen estimator and its 95% CI, with death prior to infection treated as a competing risk. The cumulative incidence of treated CMV will similarly be estimated at Day 90 for each arm.

5.6.12 Incidence of Toxicities

The frequency of Grade 3-5 toxicities per CTCAE Version 5 occurring from randomization until 56 days post-randomization will be tabulated by organ system for each treatment arm. The maximum severity of reported toxicities during that period will also be summarized. The proportions of participants reporting Grade 3-5 toxicities through Day 56 will be compared between arms using logistic regression with adjustment for the stratification factors and for acute GVHD grade at randomization (Grade III vs. IV).

5.6.13 Subgroup Analyses of Day 28 CR Rate

In a secondary analysis of the primary endpoint, the CR rate at Day 28 post-randomization will be described for each age group (55 years or over vs. under 55), gender, race, ethnicity, center region (US vs. Europe), and aGVHD grade (III vs. IV). Sample proportions and 95% Wilson score or Clopper-Pearson CIs will estimate the Day 28 CR rate of each subgroup by treatment arm. For each subgroup, the difference in CR rates between arms will be described using a point estimate and 95% CI.

5.7 ANALYSIS OF PHARMACOKINETIC / PHARMACODYNAMIC ENDPOINTS (T-GUARD ONLY)

The analysis of pharmacokinetic / pharmacodynamic endpoints will be based on the PK analysis population.

5.7.1 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 (see Table K-1). The model will be used to evaluate the following metrics:

- C_{inf}: 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.

5.7.2 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 6, 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.

5.8 ANALYSIS OF EXPLORATORY ENDPOINTS

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

5.8.1 Discontinuation of Systemic Steroids

The proportion of participants that is free of systemic steroids at Day 180 post-randomization will be described by treatment arm using sample proportions and 95% Wilson score or Clopper-Pearson CIs. The proportions of steroid-free participants at Day 180 will be compared between arms using Barnard's exact test.

5.8.2 CMV Reactivation

Among participants who were CMV positive at enrollment, the cumulative incidence of initiation of systemic treatment for CMV-reactivation will be described for each treatment arm using the Aalen-Johansen estimator, with death treated as a competing risk. Estimates and 95% CIs of the cumulative incidence of CMV reactivation will be provided at Day 180 post-randomization. The cumulative incidence of CMV will be compared between arms using Gray's test.

5.8.3 EBV-associated Lymphoproliferative Disorder

The cumulative incidence of either EBV-associated lymphoproliferative disorder or EBV reactivation requiring therapy with rituximab will be described for each treatment arm using the Aalen-Johansen estimator, with death treated as a competing risk. Estimates and 95% CIs of the cumulative incidence of EBV-associated lymphoproliferative disorder/EBV reactivation will be provided at Day 180 post-randomization. The cumulative incidence of EBV-associated lymphoproliferative disorder/EBV reactivation will be compared between arms using Gray's test.

5.8.4 Evolution of Cell Populations

The evolution of specific cell populations over the 180-day follow-up period will be evaluated using samples obtained from approximately 50 participants (25 T-Guard and 25 ruxolitinib participants) at Days 0, 14, 28, 90 and 180 post-randomization. Specimens will either be collected and stored as viably frozen PBMCs to allow for phenotypic and functional analysis of specific cell subsets or collected in CytoChex blood preservation tubes for FCM analysis.

The following cell populations, amongst others, 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.

The level of each cell population will be summarized for each arm using descriptive statistics at each assessment time considered. Changes in levels from baseline to each follow-up assessment time will be evaluated by arm using Wilcoxon signed rank tests. For the PBMC subsets collected from both arms, Wilcoxon rank sum tests will compare the changes between arms at each follow-up time point.

5.8.5 GVHD-related Biomarkers

Using the biomarker risk model from Major-Monfried, Renteria et al. 2018, the serum levels of GVHD-related biomarkers including REG3α and ST2 and the 3-IS urine levels at enrollment and Day 6, 14, and 28 post-randomization will be used to estimate NRM probabilities for each participant at Day 180 following each assessment time point. The proportions of participants with high risk (defined as an estimated NRM greater than 0.29) will be described at each assessment time by treatment arm.

Moreover, the proportions of participants within each treatment arm experiencing a Day 28 CR will be described separately for participants with high risk vs. not at enrollment within each treatment arm using sample proportions and 95% Wilson score or Clopper-Pearson CIs, as appropriate. A logistic regression model will evaluate the prognostic value of biomarker risk (high vs. not) on the chance of attaining a Day 28 CR while adjusting for treatment arm.

5.8.6 Patient Reported Outcomes (PROs)

Patient self-reported measures will be assessed using selected PROMIS domains for gastrointestinal symptoms, physical function, and satisfaction with participation in social roles, described in APPENDIX E. These instruments will be scored according to the recommendation of the developers. For each PROMIS domain, scores at baseline and at Day 28, 90, and 180

post-randomization will be described by treatment arm using descriptive statistics. Wilcoxon signed rank tests will be used to evaluate changes from baseline to each follow-up time point for each arm. Wilcoxon rank sum tests will compare the changes between arms at each of these time points.

5.8.7 Thrombotic Microangiopathy (TMA)

The frequency of TMA events will be tabulated for each treatment arm. The cumulative incidence of TMA will be described by arm using the Aalen-Johansen estimator, with death treated as a competing risk. Estimates and 95% CIs of the cumulative incidence of TMA will be provided at Day 28 post-randomization. The cumulative incidence of TMA will be compared between arms using Gray's test.

5.8.8 EASIX Score

EASIX score at enrollment will be summarized by treatment arm using descriptive statistics. The proportion of participants with high EASIX OS risk (defined as an EASIX score greater than 3.43) and high EASIX NRM risk (EASIX score greater than 3.61) at the time of enrollment will also be described for each arm, the risk classifications proposed in Luft et al. 2017.

Validation of these EASIX risk categories will be performed in the context of SR-aGVHD treatment considered in the current trial. OS and NRM at Day 180 post-randomization will be estimated for each EASIX risk group (high vs. not) by treatment arm using the Kaplan-Meier and Aalen-Johansen estimators, respectively. A Cox proportional hazards model will evaluate the prognostic value of EASIX OS risk (high vs. not) on the hazard rate of overall mortality while adjusting for treatment arm and a EASIX risk-treatment arm interaction term to permit possible differential effects of the treatments based on EASIX classification. A similar Cox model of the cause-specific hazard rate of NRM will evaluate the prognostic value of EASIX NRM risk (high vs. not) while adjusting for treatment arm and a EASIX risk-treatment arm interaction term.

APPENDIX A: HUMAN SUBJECTS

1. Subject Consent

Candidates for the study will be identified as described in Chapter 4 of the protocol. The PI or his/her designee at each transplant center will contact the candidates, provide the participant 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 participant 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, and other populations will be included in this study. Children 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 aGVHD 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 2002 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 study treatment
- Evaluation of serum and urine biomarkers associated with aGVHD
- PK of T-Guard - development of a population pharmacokinetic model
- Evaluation of potential humoral responses (ADA) to T-Guard infusions
- Evaluation of study treatment on targeted and non-target immune cell subsets and presence of alloreactive and CMV/EBV specific T-cells
- Evaluation of free-mAb and free-RTA in serum in T-Guard treated patients

Once the samples are collected at specified time points, they will either be shipped on the day of collection or aliquoted and stored on site until batch-shipping to the specified lab. The collection and shipment of these blood and urine samples will be tracked. Detailed procedures regarding specimen collection schedules, sample handling/processing procedures and shipping instructions will be found in the BMT CTN 2002 Research Sample Information Guide.

Subjects	Sample Type	Sample Collection Time Points	Sample Collection and Sample Processing Summary	Shipping Specifications
T-Guard Participants Only	**Peripheral Blood** (SST Clot Tube) **5 mL** **Humoral Response Serum Anti-Drug Antibodies**	**Pre-treatment initiation** Day 0 **Post-treatment initiation** Day 6 (just prior to 4th infusion) Days 14, 28, 90, and 180	Collect the blood sample and place into an SST Vacutainer tube containing clot activator. Allow blood samples to clot upright for 30-60 minutes in a tube rack prior to centrifugation. In Europe: Samples should be aliquoted and stored at -80ºC.	US: Centrifuged blood sample tube will be shipped at 2-8ºC on the day of collection, to the BMT CTN Biorepository, aliquoted, and stored at -80ºC. The sample aliquots will be batch-shipped to Ardena for analysis. Europe: Frozen serum aliquots will be periodically batched-shipped to Ardena for analysis.
T-Guard Participants Only	**Peripheral Blood**[1] (SST Clot Tube) **5 mL** **Serum Pharmacokinetic Testing (2 aliquots)** **Cytokines testing during safety run-in and for patients experiencing an infusion reaction (1 aliquot)** **Free-RTA + free-mAb testing on selected left-over PK samples (1 aliquot)**	**Day 0 (1st 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 and 24 hours after start of infusion **Day 6 (4th infusion)** Prior to study drug infusion ***and*** 4, 6, 24, and 48 hours after start of infusion **Notably no PK sampling is required on Weekend Days**	Collect the blood sample and place into an SST Vacutainer tube containing clot activator. Allow blood samples to clot upright for 30-60 minutes in a tube rack prior to centrifugation, serum removal, and serum aliquot equally in 4 aliquots of 0.5 mL; storage at -80ºC.	Frozen serum aliquots will be periodically batched-shipped to the BMT CTN Biorepository (US) or Ardena (Europe). One aliquot will be shipped from BMT CTN/Ardena to MyriadRBM for cytokine testing for the first 12 T-Guard patients, and in cases where patients experience a T-Guard infusion reaction. All other sample aliquots wil be PK tested at Ardena. Remaining PK samples may be selected for free-RTA + free-mAb testing.
All Study Participants	**Peripheral Blood** (SST Clot Tube) **5 mL** **Serum GVHD Biomarkers REG3α and ST2**	**Pre-treatment initiation** Day 0 **Post-treatment initiation** Day 6 (just prior to 4th infusion for T-Guard arm) Days 14 and 28	Collect the blood sample and place 5 mL SST Vacutainer tube containing clot activator. Allow blood samples to clot upright for 30-60 minutes in a tube rack prior to centrifugation. In Europe: Samples should be aliquoted and stored at -80ºC.	US: Centrifuged blood sample tube will be shipped at 2-8ºC on the day of collection, to the BMT CTN Biorepository and stored at -80ºC. Serum aliquots will be batch-shipped to Dr. Ferrara's lab at Mt. Sinai for analysis. Europe: Frozen serum aliquots will be batched-shipped to Ardena, and forwarded Dr. Holler's lab in Regensburg, Germany for analysis.

Subjects	Sample Type	Sample Collection Time Points	Sample Collection and Sample Processing Summary	Shipping Specifications
All Study Participants	**Urine** **6 mL** **GVHD Related 3IS Biomarkers**	**Pre-treatment initiation** Day 0 **Post- treatment initiation** Day 6 (just prior to 4th infusion for T-Guard arm) Days 14 and 28	In the US: Place urine in sterile transport tube for same-day shipping. In Europe: Aliquote urine and place in ultra-freezer temperature cryovials and stored at -80ºC.	US: Urine sample tube will be shipped at 2-8ºC on the day of collection to the BMT CTN Biorepository, aliquoted, and stored at -80ºC. Europe: Frozen aliquots will be periodically batched-shipped to Ardena, and forwarded to Dr. Holler's lab in Regensburg, Germany for analysis.
50 Study Participants in selected centers (25 on T-Guard and 25 on ruxolitinib)	**Peripheral Blood** (Sodium Heparin Vacutainer Tubes) **30 mL** **Peripheral blood mononuclear cells (PBMC) viably frozen**	**Pre-treatment initiation** Day 0 **Post-treatment initiation** Days 14, 28, 56, 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. In Europe: Samples will be viably frozen at site.	US: Blood sample tubes will be shipped at 2-8ºC on the day of collection, to the BMT CTN Biorepository for viably freezing. Europe: Samples should be viably frozen at site and batch-shipped to Ardena. Samples will be banked for later analysis (e.g. CMV/EBV Alloreactive T-Cells).
50 Study Participants in selected centers (25 on T-Guard and 25 on ruxolitinib)	**Peripheral Blood** (Cyto-Chex BCT) **10 mL** **Flow Cytometry Immune Profiling**	**Pre-treatment initiation** Day 0 **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 (US) or Ardena (Europe).

[1]Pharmacokinetic blood samples directly at pre-infusion and at 4, 5, 6, 8, and 24 hours after start of 1st infusion; pre-infusion and at 4 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 is allowed for samples drawn at 4 hours, a window of +/- 30 minutes for samples drawn at 5, 6 or 8 hours after the start of infusion, and a window of +/- 60 minutes for the sample drawn at 24 and 48 hours after the start of infusion. Every effort should be made to collect PK samples at all timepoints; however, ***no*** PK sampling is required on weekend days.

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 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):*				

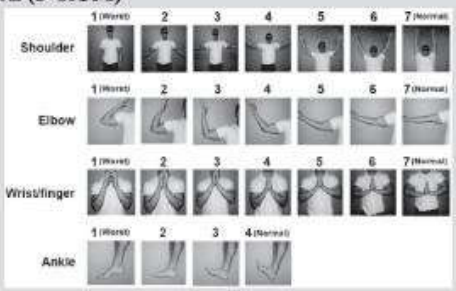
	SCORE 0	SCORE 1	SCORE 2	SCORE 3
JOINTS AND FASCIA P-ROM score *(see below)* Shoulder (1-7):___ Elbow (1-7): ____ Wrist/finger (1-7):___ Ankle (1-4):___	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs **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 general, would you say your quality of life is….

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

In general, how would you rate your physical health?

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

In general, how would you rate your mental health, including your mood and your ability to think?

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

In general, how would you rate your satisfaction with your social activites and relationships?

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

In general, please rate how well you carry out your usual social activities and roles. (This includes activities at home, at work and in your community, and responsibilities as a parent, child, spouse, employee, friend, etc.)

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

To what extent are you able to carry out your everyday physical activities such as walking, climbing stairs, carrying groceries ro moving a chair?

1. Not at all
2. A little
3. Moderately
4. Mostly
5. Completely

How often have you been bothered by emotional problems such as feeling anxious, depressed or irritable?

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

How would you rate your fatigue on average?

1. Very severe
2. Severe
3. Moderate
4. Mild
5. None

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 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, 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: DERIVATION OF A SEQUENTIAL TEST FOR COMPARING BINOMIAL PROPORTIONS BETWEEN TWO STUDY ARMS

Background – The Sequential Probability Ratio Test

Let $f(.,\theta)$ be the density function for a random variable X. According to Neyman and Pearson, the most powerful test of H_0: $\theta = \theta_o$ versus H_1: $\theta = \theta_1$ decides in favor of H_1 or H_0 if $L_n > c_\alpha$ or $L_n < c_\alpha$, respectively, where $L_n = \prod_i^n f(x_i;\theta_1)/f(x_i;\theta_0)$ is the likelihood ratio, and c_α is the critical value determine so that the test will have size α. When the sample size is not fixed in advance, further improvement is possible by using Wald's Sequential Probability Ratio Test (SPRT). The SPRT continues to sample as long as $B < L_n < A$ for some constants A, B with $B < 1 < A$, stops sampling and decides in favor of H_1 as soon as $L_n > A$, and stops sampling and decides in favor of H_0 as soon as $L_n < B$.

The usual measures of performance of such a procedure are the error probabilities α and β of rejecting H_0 when $\theta = \theta_0$ and of accepting H_0 when $\theta = \theta_1$, respectively, and the expected sample size $E(N|\theta_j) = E_j(N)$. Wald and Wolfowitz showed that among all tests, sequential or not, for which $P($ reject $H_0|\theta = \theta_0) \leq \alpha$, $P($ accept $H_0|\theta = \theta_1) \leq \beta$, and the $E_j(N)$ are finite for $j = 0,1$, the SPRT with error probabilities α and β minimizes $E_0(N)$ and $E_1(N)$. If, in addition, the x_i are independent and identically distributed (i.i.d.) with density function $f(x,\theta)$, with monotone likelihood ratio in $\tau(x)$, then any SPRT for testing θ_0 against $\theta_1 (> \theta_0)$ has a non-decreasing power function.

For the SPRT with error probabilities α and β, the SPRT boundaries are given approximately by $A = (1-\beta)/\alpha$ and $B = \beta/(1-\alpha)$. The operating characteristics of the SPRT are given by $O(\theta,\alpha,\beta,\theta_0,\theta_1) = (A^{h(\theta)} - 1)/(A^{h(\theta)} - B^{h(\theta)})$ where $h(\theta)$ is the non-trivial solution to the equation $\int [f(x;\theta_1)/f(x,\theta_2)]^{h(\theta)} f(x;\theta)dx = 1$.

The formula $E(N;\theta) = \{[(1\{O(\theta)]\, log\, A + O(\theta)\, log\, B\}/E(z;\theta)$ provides the average sample number for an arbitrary θ. The sample size distribution is highly skewed with $Var(N) \approx [E(N)]^2$. Thus, we consider a truncated test with maximum sample size of N_0 and simulate to obtain the operating characteristics of the test.

Derivation of the SPRT for Comparing Binomial Proportions Between Two Arms

Suppose that we wish to construct a sequential test that compares the proportions of participants experiencing an event of interest between two treatment arms, Arm 1 and Arm 2, with interest lying in detecting excess event risk in Arm 1 if it exists. This is equivalent to comparing the composite null hypothesis that the odds ratio θ of Arm 1 to Arm 2 equals 1 versus the alternative hypothesis that $\theta > 1$.

At a given point during the study, let n denote the current number of participants who are evaluable for the event's occurrence, n_i be the current number of evaluable participants on treatment arm i, x_{ij} be a binary indicator of whether the j^{th} participant on treatment arm i had an event, and $y_i = \sum_{j=1}^{n_i} x_{ij}$ be the total number of events observed in treatment arm i. If we condition on the total number of events $y = y_1 + y_2$, the distribution of y_1 is known to follow Fisher's noncentral hypergeometric distribution with probability mass function

$$f(y_1|\theta,y) = \frac{\binom{n_1}{y_1}\binom{n_2}{y_2}\theta^{y_1}}{\sum_{k=\max(0,y-n_2)}^{\min(n_1,y)}\binom{n_1}{k}\binom{n_2}{y-k}\theta^k}.$$

Then the conditional likelihood of θ given y can be written as $L_c(\theta) = L(\theta|y) = f(y_1|\theta, y)$, which is a monotone function of y_1. Thus, it is straightforward to apply the SPRT toward a sequential test comparing events rates.

The SPRT can be derived with reference to point null and alternative hypotheses, $H_0: \theta = 1$ and $H_1: \theta = \theta_1$ for some value $\theta_1 > 1$ chosen to indicate unacceptably elevated risk in Arm 1 compared to Arm 2. The sequential test that we employ modifies this SPRT in two ways:

1) Only early stopping to reject H_0 is included in order to exercise enhanced vigilance in safety monitoring
2) The test is truncated at a prespecified number of evaluable participants N_0 such that, if H_0 is not rejected after all N_0 participants become evaluable, H_0 will be accepted

Therefore, our testing procedure will reject H_0 after the n^{th} participant becomes evaluable if the likelihood ratio $L_n = \frac{L_c(\theta)}{L_c(1)}$ exceeds $A = (1 - \beta)/\alpha$ for $n = 1, \ldots, N_0$. Because this is a modification of the standard SPRT, operating characteristics should be simulated to assess its performance across a range of values for the true event probabilities $P(x_{ij} = 1)$ for Arms 1 and 2.

APPENDIX G: BMT CTN INFECTION GRADING TABLE AND RECURRENCE INTERVAL DEFINITIONS

Type of Infection/ Severity Grade	Grade 1	Grade 2	Grade 3
Bacterial infections	Bacterial focus NOS requiring no more than 14 days of therapy for treatment (e.g urinary tract infection)	Bacteremia (except CoNS) without severe sepsis ***	Bacteremia with deep organ involvement (e.g. with new or worsening pulmonary infiltrates; endocarditis)
	Coag Neg Staph (S. epi), Corynebacterium, or Proprioniobacterium bacteremia	Bacterial focus with persistent signs, symptoms or persistent positive cultures requiring greater than 14 days of therapy	Severe sepsis with bacteremia.
	Cellulitis responding to initial therapy within 14 days	Cellulitis requiring a change in therapy d/t progression Localized or diffuse infections requiring incision with or without drain placement	Fasciitis requiring debridement
		Any pneumonia documented or presumed to be bacterial	Pneumonia requiring intubation Brain abscess or meningitis without bacteremia
	C. Difficile toxin positive stool with diarrhea < 1L without abdominal pain (child < 20 mL/kg)	C. Difficile toxin positive stool with diarrhea $\geq$ 1L (child $\geq$ 20 mL/kg) or with abdominal pain	C. Difficile toxin positive stool with toxic dilatation or renal insufficiency with/without diarrhea
Fungal infections	Superficial candida infection (e.g. oral thrush, vaginal candidiasis)	Candida esophagitis (biopsy proven). Proven or probable fungal sinusistis confirmed radiologically without orbital, brain or bone involvement.	Fungemia including Candidemia Proven or probable invasive fungal infections (e.g., Aspergillus, Mucor, Fusarium, Scedosporium).

Type of Infection/ Severity Grade	Grade 1	Grade 2	Grade 3
Fungal infections continued			Disseminated infections (defined as multifocal pneumonia, presence of urinary or blood antigen, and/or CNS involvement) with Histoplasmosis, Blastomycosis, Coccidiomycosis, or Cryptococcus. *Pneumocystis jiroveci* pneumonia (regardless of PaO2 level)
Viral infections	Mucous HSV infection		
	Dermatomal Zoster	VZV infection with 3 or more dermatomes	Severe VZV infection (coagulopathy or organ involvement)
	Asymptomatic CMV viremia untreated or a CMV viremia with viral load decline by at least 2/3 of the baseline value after 2 weeks of therapy	Clinically active CMV infection (e.g. symptoms, cytopenias) or CMV Viremia not decreasing by at least 2/3 of the baseline value after 2 weeks of therapy	CMV end-organ involvement (pneumonitis, enteritis, retinitis)
	EBV reactivation not treated with rituximab	EBV reactivation requiring institution of therapy with rituximab	EBV PTLD
	Adenoviral conjunctivitis asymptomatic viruria, asymptomatic stool shedding and viremia not requiring treatment	Adenoviral upper respiratory infection, viremia, or symptomatic viruria requiring treatment	Adenovirus with end-organ involvement (except conjunctivitis and upper respiratory tract)
	Asymptomatic HHV-6 viremia untreated or an HHV-6 viremia with a viral load decline by at least 0.5 log after 2 weeks of therapy	Clinically active HHV-6 infection (e.g. symptoms, cytopenias) or HHV-6 viremia without viral load decline 0.5 log after 2 weeks of therapy	
	BK viremia or viruria with cystitis not requiring intervention	BK viremia or viruia with clinical consequence requiring prolonged therapy and/or surgical intervention	
		Enterocolitis with enteric viruses	

Type of Infection/ Severity Grade	Grade 1	Grade 2	Grade 3
Viral infections continued		Symptomatic upper tract respiratory virus	Lower tract respiratory viruses
	Viremia (virus not otherwise specified) not requiring therapy	Any viremia (virus not otherwise specified) requiring therapy	
			Any viral encephalitis or meningitis
Parasitic infections			CNS or other organ toxoplasmosis Strongyloides hyperinfection
Nonmicrobiologically defined infections	Uncomplicated fever with negative cultures responding within 14 days		
	Clinically documented infection not requiring inpatient management	Pneumonia or bronchopneumonia not requiring mechanical ventilation	Any acute pneumonia requiring mechanical ventilation
		Typhlitis	
			Severe sepsis*** without an identified organism

*Concomitant or multimicrobial infections are graded according to the grade of the infection with the higher grade of severity.

**Therapy includes both PO and IV formulations

***Severe Sepsis:

Adults:

Hypotension

-A systolic blood pressure of <90 mm Hg or a reduction of >40 mm hg from baseline in the absence of other causes for hypotension

Multiple Organ Dysfunction Syndrome

-2 or more of the following: Renal failure requiring dialysis, respiratory failure requiring bipap or intubation, heart failure requiring pressors, liver failure

Disseminated Infections:

1. Two or more non-contiguous sites with the SAME organism
2. A disseminated infection can occur at any level of severity, but most will be grade 2 or 3.

Recurrence Intervals to Determine Whether an Infection is the Same or New:

1. CMV, HSV, EBV, HHV6: 2 months (< 60 days)
2. VZV, HZV: 2 weeks (< 14 days)
3. Bacterial, non-C. difficile: 1 week (< 7 days)
4. Bacterial, C. difficile: 1 month (< 30 days)
5. Yeast: 2 weeks (< 14 days)
6. Molds: 3 months (< 90 days)
7. Helicobacter: 1 year (< 365 days)
8. Adenovirus, Enterovirus, Influenza, RSV, Parainfluenza, Rhinovirus: 2 weeks (< 14 days)
9. Polyomavirus (BK virus): 2 months (< 60 days)

For infections coded as “Disseminated” per the *Infection Form*, any previous infection with the same organism but different site within the recurrence interval for that organism will be counted as part of the disseminated infection.

APPENDIX H: LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AAT	Alpha-1 Antitrypsin
ABW	Actual Body Weight
ADA	Anti-Drug-Antibody
AE	Adverse Event
AIBW	Adjusted Ideal Body Weight
Allo-HSCT	Allogeneic Hematopoietic Stem Cell Transplantation
ATG	Anti-Thymocyte Globulin
AUC	Areas Under the Time-Concentration Curves
B-cells	B Lymphocytes
BAT	Best Available Therapy
BSA	Body Surface Area
BMI	Body Mass Index
Cmax	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
CNI	Calcineurin Inhibitor
CR	Complete Response, the disappearance of symptoms in all organ systems
CRA	Clinical Research Associate
CRO	Clinical Research Organization
CRS	Cytokine Release Syndrome
CTCAE	Common Terminology Criteria for Adverse Events
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
FDG	Fluoro-deoxyglucose
GCP	Good Clinical Practice
GI	Gastro-intestinal
GVHD	Graft-Versus-Host Disease
aGVHD	(acute) Graft-Versus-Host Disease
cGVHD	(chronic) Graft-Versus-Host Disease

List of Abbreviations and Definitions of Terms

SR-aGVHD	Steroid-refractory (acute) Graft-Versus-Host Disease
HCT	Hematopoietic Cell Transplantation
IBW	Ideal Body Weight
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Institutional Ethics Committee
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IRB	Institutional Review Board
ITT	Intention-To-Treat
JAK	Janus Kinase
mAb	Monoclonal Antibody
MMF	Mycophenolate Mofetil
MOP	Manual of Procedures
MTX	Methotrexate
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
PI	Principal Investigator
PK	Pharmacokinetics
PP	Per Protocol
PR	Partial Response, the improvement of 1 or more organs, with no worsening in other organs
PRO	Patient Reported Outcomes
Progression/Malignancy Relapse	The time from the date of the start of treatment to the date of hematologic malignancy relapse/progression.
REG3α	Regenerating Family Member 3 Alpha
RFS	Relapse Free Survival
RTA	Ricin Toxin A-chain
SAE	Serious Adverse Event
SCTOD	Stem Cell Transplant Outcomes Database
SoC	Standard of Care
SOS/VOD	Sinusoidal obstruction syndrome/veno-occlusive disease

List of Abbreviations and Definitions of Terms

SPRT	Sequential Probability Ratio Test
SPV-T3a	Anti-CD3, IgG2b
SR	Steroid-refractory
ST2	Interleukin 1 receptor-like 1
$t_{1/2}$	Terminal-phase Elimination Half-life
T cells	T Lymphocytes
TCR	T-cell Receptor
TEAE	Treatment Emergent Adverse Event
TMA	Thrombotic Microangiopathy
WT1	Anti-CD7, IgG2a
3-IS	3-Indoxyl Sulfate

APPENDIX I: REFERENCES

Aandahl, E. M., M. F. Quigley, W. J. Moretto, M. Moll, V. D. Gonzalez, A. Sonnerborg, S. Lindback, F. M. Hecht, S. G. Deeks, M. G. Rosenberg, D. F. Nixon and J. K. Sandberg (2004). "Expansion of CD7(low) and CD7(negative) CD8 T-cell effector subsets in HIV-1 infection: correlation with antigenic load and reversion by antiretroviral treatment." Blood 104(12): 3672-3678.

Aandahl, E. M., J. K. Sandberg, K. P. Beckerman, K. Tasken, W. J. Moretto and D. F. Nixon (2003). "CD7 is a differentiation marker that identifies multiple CD8 T cell effector subsets." J Immunol 170(5): 2349-2355.

Akbar, A. N., P. L. Amlot, K. Ivory, A. Timms and G. Janossy (1990). "Inhibition of alloresponsive naive and memory T cells by CD7 and CD25 antibodies and by cyclosporine." Transplantation 50(5): 823-829.

Ali, H., Snyder, D., Stiller, T., Synold, T., Mokhtari, S., Palmer, J., Salhotra, A., Pullarkat, V. A., Cai, J., Forman, S. J., Mei, M., Nakamura, R., "Peri-Transplant Administration of Ruxolitinib Is Safe and Feasible in Patients with Myelofibrosis: Primary Results of a Pilot Open-Label Study of Ruxolitinib Administration in Combination with Reduced Intensity Conditioning." Blood 2019; 134 (Supplement_1): 669

Alousi, A. M., D. J. Weisdorf, B. R. Logan, J. Bolanos-Meade, S. Carter, N. Difronzo, M. Pasquini, S. C. Goldstein, V. T. Ho, B. Hayes-Lattin, J. R. Wingard, M. M. Horowitz and J. E. Levine (2009). "Etanercept, mycophenolate, denileukin, or pentostatin plus corticosteroids for acute graft-versus-host disease: a randomized phase 2 trial from the Blood and Marrow Transplant Clinical Trials Network." Blood 114(3): 511-517.

Amlot, P. L., M. J. Stone, D. Cunningham, J. Fay, J. Newman, R. Collins, R. May, M. McCarthy, J. Richardson, V. Ghetie and et al. (1993). "A phase I study of an anti-CD22-deglycosylated ricin A chain immunotoxin in the treatment of B-cell lymphomas resistant to conventional therapy." Blood 82(9): 2624-2633.

Andersen PK, Borgan Ø, Gill RD, Keiding N. (1993) "Statistical Models Based on Counting Processes." NY Springer.

Amlot, P. L., F. Tahami, D. Chinn and E. Rawlings (1996). "Activation antigen expression on human T cells. I. Analysis by two-colour flow cytometry of umbilical cord blood, adult blood and lymphoid tissue." Clin Exp Immunol 105(1): 176-182.

Anasetti, C., P. J. Martin, R. Storb, F. R. Appelbaum, P. G. Beatty, J. Davis, K. Doney, H. F. Hill, P. Stewart, K. M. Sullivan and et al. (1992). "Treatment of acute graft-versus-host disease with a nonmitogenic anti-CD3 monoclonal antibody." Transplantation 54(5): 844-851.

Appay, V., R. A. van Lier, F. Sallusto and M. Roederer (2008). "Phenotype and function of human T lymphocyte subsets: consensus and issues." Cytometry A 73(11): 975-983.

Bacigalupo, A. (2007). "Management of acute graft-versus-host disease." Br J Haematol 137(2): 87- 98.

Bacigalupo, A., M. T. van Lint, F. Frassoni, M. Podesta, G. Veneziano, G. Avanzi, V. Vitale and A. M. Marmont (1983). "High dose bolus methylprednisolone for the treatment of acute graft versus host disease." Blut 46(3): 125-132.

Bolanos-Meade, J. and G. Vogelsang (2004). "Acute graft-versus-host-diseas. ." Clinical Advances in Hematology & Oncology. 2(10): 672-682.

Byers, V. S., P. J. Henslee, N. A. Kernan, B. R. Blazar, R. Gingrich, G. L. Phillips, C. F. LeMaistre, G. Gilliland, J. H. Antin, P. Martin and et al. (1990). "Use of an anti-pan T-lymphocyte ricin a chain immunotoxin in steroid-resistant acute graft-versus-host disease." Blood 75(7): 1426-1432.

Castilla-Llorente, C., P. J. Martin and G. McDonald (2014). "Prognostic factors and outcomes of severe gastrointestinal GVHD after allogeneic hematopoietic cell transplantation." Bone Marrow Transplant 49: 966-971.

Deeg, H. J. (2007). "How I treat refractory acute GVHD." Blood 109(10): 4119-4126.

Derocq, J. M., G. Laurent, P. Casellas, H. Vidal, P. Poncelet, A. Fauser, C. Demur and F. Jansen (1987). "Rationale for the selection of ricin A-chain anti-T immunotoxins for mature T cell depletion." Transplantation 44(6): 763-769.

Engert, A., V. Diehl, R. Schnell, A. Radszuhn, M. T. Hatwig, S. Drillich, G. Schon, H. Bohlen, H. Tesch, M. L. Hansmann, S. Barth, J. Schindler, V. Ghetie, J. Uhr and E. Vitetta (1997). "A phase-I study of an anti-CD25 ricin A-chain immunotoxin (RFT5-SMPT-dgA) in patients with refractory Hodgkin's lymphoma." Blood 89(2): 403-410.

Filipovich, A. H., D. Weisdorf, S. Pavletic, G. Socie, J. R. Wingard, S. J. Lee, P. Martin, J. Chien, D. Przepiorka, D. Couriel, E. W. Cowen, P. Dinndorf, A. Farrell, R. Hartzman, J. Henslee-Downey, D. Jacobsohn, G. McDonald, B. Mittleman, J. D. Rizzo, M. Robinson, M. Schubert, K. Schultz, H. Shulman, M. Turner, G. Vogelsang and M. E. Flowers (2005). "National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report." Biol Blood Marrow Transplant 11(12): 945-956.

Floisand, Y., K. E. A. Lundin, V. Lazarevic, J. D. Kristiansen, L. T. N. Osnes, G. E. Tjonnfjord, H. M. Reims and T. Gedde-Dahl (2017). "Targeting Integrin alpha4beta7 in Steroid-Refractory Intestinal Graft-versus-Host Disease." Biol Blood Marrow Transplant 23(1): 172-175.

Frenken, L. A., R. A. Koene and W. J. Tax (1991). "The role of antibody isotype in IFN-gamma and IL-2 production during anti-CD3-induced T cell proliferation." Transplantation 51(4): 881-887.

Gamadia, L. E., R. J. Rentenaar, P. A. Baars, E. B. Remmerswaal, S. Surachno, J. F. Weel, M. Toebes, T. N. Schumacher, I. J. ten Berge and R. A. van Lier (2001). "Differentiation of cytomegalovirus-specific CD8(+) T cells in healthy and immunosuppressed virus carriers." Blood 98(3): 754-761.

Gill, S., J. A. Olson and R. S. Negrin (2009). "Natural killer cells in allogeneic transplantation: effect on engraftment, graft- versus-tumor, and graft-versus-host responses." Biol Blood Marrow Transplant 15(7): 765-776.

Groth, C., L. F. van Groningen, M. E. J. Bremmers, F. Preijers, H. Dolstra, T. R. Matos, C. Reichterts, H. G. van Hooren, Y. V. van Oosterhout, J. E. Levine, J. L. Ferrara, N. M. Blijlevens, M. Stelljes and W. van der Velden (2017). "A Phase I/II Study on the Anti-DC3/CD7 Immunotoxin Combination (T-GuardTM) for the Treatment of Steroid Refractory Acute GvHD." Blood 130(1): 513.

Groth, C., L. F. J. van Groningen, T. R. Matos, M. E. Bremmers, F. Preijers, H. Dolstra, C. Reicherts, N. P. M. Schaap, E. H. G. van Hooren, J. IntHout, R. Masereeuw, M. G. Netea, J. E. Levine, G. Morales, J. L. Ferrara, N. M. A. Blijlevens, Y. van Oosterhout, M. Stelljes and W. van der Velden (2018). "Phase I/II Trial of a Combination of Anti-CD3/CD7 Immunotoxins for Steroid-Refractory Acute Graft-versus-Host Disease." Biol Blood Marrow Transplant.

Harris, A. C., R. Young, S. Devine, W. J. Hogan, F. Ayuk, U. Bunworasate, C. Chanswangphuwana, Y. A. Efebera, E. Holler, M. Litzow, R. Ordemann, M. Qayed, A. S. Renteria, R. Reshef, M. Wolfl, Y. B. Chen, S. Goldstein, M. Jagasia, F. Locatelli, S. Mielke, D. Porter, T. Schechter, Z. Shekhovtsova, J. L. Ferrara and J. E. Levine (2016). "International, Multicenter Standardization of Acute Graft- versus-Host Disease Clinical Data Collection: A Report from the Mount Sinai Acute GVHD International Consortium." Biol Blood Marrow Transplant 22(1): 4-10.

Heinrich, G., H. Gram, H. P. Kocher, M. H. Schreier, B. Ryffel, A. Akbar, P. L. Amlot and G. Janossy (1989). "Characterization of a human T cell-specific chimeric antibody (CD7) with human constant and mouse variable regions." J Immunol 143(11): 3589-3597.

Herrera, L., R. A. Farah, V. A. Pellegrini, D. B. Aquino, E. S. Sandler, G. R. Buchanan and E. S. Vitetta (2000). "Immunotoxins against CD19 and CD22 are effective in killing precursor-B acute lymphoblastic leukemia cells in vitro." Leukemia 14(5): 853-858.

Hill, L., A. Alousi, P. Kebriaei, R. Mehta, K. Rezvani and E. Sphall (2018). "New and emerging therapies for acute and chronic graft versus host disease." Ther Adv Hematol 9(1): 21-46.

Hings, I. M., R. Severson, A. H. Filipovich, B. R. Blazar, J. H. Kersey, N. K. Ramsay, P. B. McGlave and D. J. Weisdorf (1994). "Treatment of moderate and severe acute GVHD after allogeneic bone marrow transplantation." Transplantation 58(4): 437-442.

Ho, V. T., D. Zahrieh, E. Hochberg, E. Micale, J. Levin, C. Reynolds, S. Steckel, C. Cutler, D. C. Fisher, S. J. Lee, E. P. Alyea, J. Ritz, R. J. Soiffer and J. H. Antin (2004). "Safety and efficacy of denileukin diftitox in patients with steroid-refractory acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation." Blood 104(4): 1224-1226.

Holtan SG, P. M., Weisdorf DJ. (2014). "Acute graft-versus-host disease: a bench-to-bedside update." Blood 124: 363-373.

Jagasia, M., H. T. Greinix, M. Arora, K. M. Williams, D. Wolff and E. W. Cowen (2015). "Development Project on Criteria for Clinical Trials in Chronic Graft-Versus-Host Diseas: I. The 2014 Diagnosis and Staging Working Group report. ." Biol Blood Marrow Transplant 21: 389-401.

Jagasia, M., R. Zeiser, M. Arbushites, P. Delaite, B. Gadbaw and N. V. Bubnoff (2018). "Ruxolitinib for the treatment of patients with steroid-refractory GVHD: an introduction to the REACH trials." Immunotherapy 10(5): 391-402.

Jagasia, M. H., H. T. Greinix, M. Arora, K. M. Williams, D. Wolff, E. W. Cowen, J. Palmer, D. Weisdorf, N. S. Treister, G. S. Cheng, H. Kerr, P. Stratton, R. F. Duarte, G. B. McDonald, Y. Inamoto, A. Vigorito, S. Arai, M. B. Datiles, D. Jacobsohn, T. Heller, C. L. Kitko, S. A. Mitchell, P. J. Martin, H. Shulman,
R. S. Wu, C. S. Cutler, G. B. Vogelsang, S. J. Lee, S. Z. Pavletic and M. E. Flowers (2015). "National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report." Biol Blood Marrow Transplant 21(3): 389-401 e381.

Kahan B.C., Morris T.P., (2012). “Improper analysis of trials randomised using stratified blocks or minimisation. Statistics in medicine.” Stat Med. 31(4):328-40

Knight, R. J., R. Kurrle, J. McClain, J. Racenberg, V. Baghdahsarian, R. Kerman, R. Lewis, C. T. van Buren and B. D. Kahan (1994). "Clinical evaluation of induction immunosuppression with a murine IgG2b monoclonal antibody (BMA 031) directed toward the human alpha/beta-T cell receptor." Transplantation 57(11): 1581-1588.

Koyama T, Chen H. (2008) “Proper inference from Simon's two-stage designs.” Statistics in medicine. 27(16):3145-54.

Krance, R., H. E. Heslop, H. Mahmoud, R. Ribeiro, E. Douglass, C. Hurwitz, V. Santana, L. Kun, M. M. Horowitz and M. K. Brenner (1993). "Anti-pan T lymphocyte ricin A chain immunotoxin (H65-RTA) and methylprednisolone for acute GVHD prophylaxis following allogeneic BMT from HLA-identical sibling donors." Bone Marrow Transplant 11(1): 33-36.

Land, W., G. Hillebrand, W. D. Illner, D. Abendroth, E. Hancke, S. Schleibner, C. Hammer and J. Racenberg (1988). "First clinical experience with a new TCR/CD3-monoclonal antibody (BMA 031) in kidney transplant patients." Transpl Int 1(2): 116-117.

LaQuisa, H. A., A.; Partow, K.; Rohtesh, M.; Katayou, R.; Shpall, E. (2018). "New and emerging therapies for acute and chronic graft versus host disease." Ther Adv Hematol 9(1): 21-46.

Levine, J. E., B. Logan, J. Wu, A. M. Alousi, V. Ho, J. Bolanos-Meade and D. Weisdorf (2010). "Graft-versus-host disease treatment: predictors of survival." Biol Blood Marrow Transplant 16(12): 1693-1699.

Luft T, Benner A, Jodele S, Dandoy CE, Storb R, Gooley T, Sandmaier BM, Becker N, Radujkovic A, Dreger P, Penack O. EASIX in patients with acute graft-versus-host disease: a retrospective cohort analysis. The Lancet Haematology. 2017 Sep 1;4(9):e414-23.

MacMillan, M. L., M. Robin and A. C. Harris (2015). "A refined risk score for acute graft-versus-host disease that predicts response to initial therapy, survival, and transplant-related mortality." Biol Blood Marrow Transplant 21: 761-767.

MacMillan, M. L., D. Weisdorf, J. E. Wagner, T. E. DeFor, L. J. Burns, A. Ramsay, S. M. Davies and B. Blazar (2002). "Response of 443 Patients to Steroids as Primary Therapy for Acute Graft-versus-Host Disease." Biol Blood Marrow Transplant 8: 387-394.

Magenau, J., L. Runaas and P. Reddy (2016). "Advances in understanding the pathogenesis of graft-versus-host disease." Br J Haematol 173: 190-205.

Major-Monfried, H., A. S. Renteria, A. Pawarode, P. Reddy, F. Ayuk, E. Holler, Y. A. Efebera, W. J. Hogan, M. Wolfl, M. Qayed, E. O. Hexner, K. Wudhikarn, R. Ordemann, R. Young, J. Shah, M. J. Hartwell, M. Chaudhry, M. Aziz, A. Etra, G. A. Yanik, N. Kroger, D. Weber, Y. B. Chen, R. Nakamura, W. Rosler, C. L. Kitko, A. C. Harris, M. Pulsipher, R. Reshef, S. Kowalyk, G. Morales, I. Torres, U. Ozbek, J. L. M. Ferrara and J. E. Levine (2018). "MAGIC biomarkers predict long term outcomes for steroid-resistant acute GVHD." Blood.

Marcondes, A. M., D. Hockenbery, M. Lesnikova, C. A. Dinarello, A. Woolfrey, T. Gernsheimer, M. Loghman-Adham, D. Gelmont, B. Storer, J. A. Hansen and H. J. Deeg (2016). "Response of Steroid-Refractory Acute GVHD to alpha1-Antitrypsin." Biol Blood Marrow Transplant 22(9): 1596-1601.

Marcondes, A. M., E. Karoopongse, M. Lesnikova, D. Margineantu, T. Welte, C. A. Dinarello, D. Hockenbery, S. Janciauskiene and H. J. Deeg (2014). "alpha-1-Antitrypsin (AAT)-modified donor cells suppress GVHD but enhance the GVL effect: a role for mitochondrial bioenergetics." Blood 124(18): 2881-2891.

Martin, P. J., B. J. Nelson, F. R. Appelbaum, C. Anasetti, H. J. Deeg, J. A. Hansen, G. B. McDonald, R. A. Nash, K. M. Sullivan, R. P. Witherspoon, P. J. Scannon, N. Friedmann and R. Storb (1996). "Evaluation of a CD5-specific immunotoxin for treatment of acute graft-versus-host disease after allogeneic marrow transplantation." Blood 88(3): 824-830.

Martin, P. J., J. D. Rizzo, J. R. Wingard, K. Ballen, P. T. Curtin, C. Cutler, M. R. Litzow, Y. Nieto, B. N. Savani, J. R. Schriber, P. J. Shaughnessy, D. A. Wall and P. A. Carpenter (2012). "First- and second-line systemic treatment of acute graft-versus-host disease: recommendations of the American Society of Blood and Marrow Transplantation." Biol Blood Marrow Transplant 18(8): 1150-1163.

Martin, P. J., G. Schoch, L. Fisher, V. Byers, C. Anasetti, F. R. Appelbaum, P. G. Beatty, K. Doney, G. B. McDonald, J. E. Sanders and et al. (1990). "A retrospective analysis of therapy for acute graft-versus-host disease: initial treatment." Blood 76(8): 1464-1472.

Messmann, R. A., E. S. Vitetta, D. Headlee, A. M. Senderowicz, W. D. Figg, J. Schindler, D. F. Michiel, S. Creekmore, S. M. Steinberg, D. Kohler, E. S. Jaffe, M. Stetler-Stevenson, H. Chen, V. Ghetie and E. A. Sausville (2000). "A phase I study of combination therapy with immunotoxins IgG- HD37-deglycosylated ricin A chain (dgA) and IgG-RFB4-dgA (Combotox) in patients with refractory CD19(+), CD22(+) B cell lymphoma." Clin Cancer Res 6(4): 1302-1313.

Mohty, M., Hollar, E., Jagasia, M., Malard, F., Martin, P. (2020) “Refractory acute graft-versus-host disease: a new working definition beyond corticosteroid refractoriness.” Blood 136(17):1903-1906

Paczesny, S. (2013). "Discovery and validation of graft-versus-host disease biomarkers." Blood 121(4): 585-594.

Phillips, G. L., T. J. Nevill, J. J. Spinelli, S. H. Nantel, H. G. Klingemann, M. J. Barnett, J. D. Shepherd, K. W. Chan, J. M. Meharchand, H. J. Sutherland and et al. (1995). "Prophylaxis for acute graft-versus-host disease following unrelated donor bone marrow transplantation." Bone Marrow Transplant 15(2): 213-219.

Pidala, J., Hamadani, M., Dawson, et al 2020, “Randomized multicenter trial of sirolimus vs prednisone as initial therapy for standard-risk acute GVHD: the BMT CTN 1501 trial Blood 2020” Blood 135: 2-97-107-0006-4971

Pidala, J., J. Kim, J. Perkins, T. Field, H. Fernandez, L. Perez, E. Ayala, M. Kharfan-Dabaja and C. Anasetti (2010). "Mycophenolate mofetil for the management of steroid-refractory acute graft vs host disease." Bone Marrow Transplant 45(5): 919-924.

Preijers, F. W., T. De Witte, G. P. Rijke-Schilder, W. J. Tax, J. M. Wessels, C. Haanen and P. J. Capel (1988). "Human T lymphocyte differentiation antigens as target for immunotoxins or complement-mediated cytotoxicity." Scand J Immunol 28(2): 185-194.

Preijers, F. W., W. J. Tax, T. De Witte, A. Janssen, H. vd Heijden, H. Vidal, J. M. Wessels and P. J. Capel (1988). "Relationship between internalization and cytotoxicity of ricin A-chain immunotoxins." Br J Haematol 70(3): 289-294.

Preijers, F. W., W. J. Tax, J. M. Wessels, P. J. Capel, T. De Witte and C. Haanen (1988). "Different susceptibilities of normal T cells and T cell lines to immunotoxins." Scand J Immunol 27(5): 533-540.

Reinhold, U., H. Abken, S. Kukel, M. Moll, R. Muller, I. Oltermann and H. W. Kreysel (1993). "CD7- T cells represent a subset of normal human blood lymphocytes." J Immunol 150(5): 2081-2089.

Schindler, J., S. Gajavelli, F. Ravandi, Y. Shen, S. Parekh, I. Braunchweig, S. Barta, V. Ghetie, E. Vitetta and A. Verma (2011). "A phase I study of a combination of anti-CD19 and anti-CD22 immunotoxins (Combotox) in adult patients with refractory B-lineage acute lymphoblastic leukaemia." Br J Haematol 154(4): 471-476.

Schnell, R., O. Staak, P. Borchmann, C. Schwartz, B. Matthey, H. Hansen, J. Schindler, V. Ghetie, E. S. Vitetta, V. Diehl and A. Engert (2002). "A Phase I study with an anti-CD30 ricin A-chain immunotoxin (Ki-4.dgA) in patients with refractory CD30+ Hodgkin's and non-Hodgkin's lymphoma." Clin Cancer Res 8(6): 1779-1786.

Schoemans, H. M., S. J. Lee, J. L. Ferrara, D. Woff, J. E. Levine, K. Schultz, B. E. Shaw, M. E. Flowers, T. Ruutu, H. T. Greinix, E. Holler, G. Basak, R. F. Duarte, S. Pavletic and o. b. o. t. E. T. C. W. P. a. t. E.-N.-C. G. taskforce (2018). "EBMT-NIH-CIBMTR Task Force position statement on standardized technology & guidance for graft-versus-host disease assessment." Bone Marrow Transplant.

Shaughnessy, P. J., C. Bachier, M. Grimley, C. O. Freytes, N. S. Callander, J. H. Essell, N. Flomenberg, G. Selby and C. F. Lemaistre (2005). "Denileukin diftitox for the treatment of steroid- resistant acute graft-versus-host disease." Biol Blood Marrow Transplant 11(3): 188-193.

Shindo, T., T. K. Kim, C. L. Benjamin, E. D. Wieder, R. B. Levy and K. V. Komanduri (2013). "MEK inhibitors selectively suppress alloreactivity and graft-versus-host disease in a memory stage- dependent manner." Blood 121(23): 4617-4626.

Smely, S., M. Weschka, G. Hillebrand, U. Dendorfer, F. Krombach, R. Kurrle, W. Land and C. Hammer (1990). "Prophylactic use of the new monoclonal antibody BMA 031 in clinical kidney transplantation." Transplant Proc 22(4): 1785-1786.

Stone, M. J., E. A. Sausville, J. W. Fay, D. Headlee, R. H. Collins, W. D. Figg, M. Stetler-Stevenson, V. Jain, E. S. Jaffe, D. Solomon, R. M. Lush, A. Senderowicz, V. Ghetie, J. Schindler, J. W. Uhr and E. S. Vitetta (1996). "A phase I study of bolus versus continuous infusion of the anti-CD19 immunotoxin, IgG-HD37-dgA, in patients with B-cell lymphoma." Blood 88(4): 1188-1197.

van Oosterhout, Y. V., J. L. van Emst, H. H. Bakker, F. W. Preijers, A. V. Schattenberg, D. J. Ruiter, S. Evers, J. P. Koopman and T. de Witte (2001). "Production of anti-CD3 and anti-CD7 ricin A-immunotoxins for a clinical pilot study." Int J Pharm 221(1-2): 175-186.

van Oosterhout, Y. V., L. van Emst, A. V. Schattenberg, W. J. Tax, D. J. Ruiter, H. Spits, F. M. Nagengast, R. Masereeuw, S. Evers, T. de Witte and F. W. Preijers (2000). "A combination of anti- CD3 and anti-CD7 ricin A-immunotoxins for the in vivo treatment of acute graft versus host disease." Blood 95(12): 3693-3701.

Wayne, A. S., D. J. Fitzgerald, R. J. Kreitman and I. Pastan (2014). "Immunotoxins for leukemia." Blood 123(16): 2470-2477.

Weisdorf, D., R. Haake, B. Blazar, W. Miller, P. McGlave, N. Ramsay, J. Kersey and A. Filipovich (1990). "Treatment of moderate/severe acute graft-versus-host disease after allogeneic bone marrow transplantation: an analysis of clinical risk features and outcome." Blood 75(4): 1024-1030.

Woodle, E. S., J. R. Thistlethwaite, L. K. Jolliffe, A. J. Fucello, F. P. Stuart and J. A. Bluestone (1991). "Anti-CD3 monoclonal antibody therapy. An approach toward optimization by in vitro analysis of new anti-CD3 antibodies." Transplantation 52(2): 361-368.

Zakias PD, Z. F., Zacharioudakis IM, Mylonakis E (2014). "Graft-versus-host disease prophylaxis after transplantation: a network meta-analysis." PloS one 9.

Zeiser, R., von Budnoff, N., Butler, J., Mohty, M., Niederwieser, D., Or, R., Szer, J. Wagner, E.M., Zuckerman, T., Mahuzier, B., Xu, J., Wilke, C., Gandhi, K. K., (2020) “Ruxolitinib for Glucocorticoid-Refractory Acute Graft-versus-Host Disease.” NEJMoa 382(19):1800-1810.

Zeiser, R., G. Socie and B. Blazar (2016). "Pathogenesis of acute graft-versus-host disease: from intestinal microbiota alterations to donor T cell activation." Br J Haematol 175: 191-207.

APPENDIX J: INVESTIGATOR'S SIGNATURE PAGE

A PHASE 3, RANDOMIZED, OPEN-LABEL, MULTICENTER STUDY, TO COMPARE T-GUARD TO RUXOLITINIB FOR THE TREATMENT OF PATIENTS WITH GRADE III OR IV STEROID-REFRACTORY ACUTE GRAFT-VERSUS-HOST DISEASE (SR-AGVHD)

BMT CTN PROTOCOL 2002
Version 1.0

Protocol History:

Version 1.0 ; 21May2021

I have read all pages of this clinical study protocol. I agree to conduct the study as outlined in the protocol and to comply with all the terms and conditions set out therein. I confirm that I will conduct the study in accordance with ICH GCP guidelines and applicable local regulations. I will also ensure that other relevant members of my study staff have the appropriate training and access to copies of this protocol and the ICH GCP guidelines to enable them to work in accordance with the provisions of these documents.
Principal Investigator: Signature: ---------- Date (DD Mmm YYYY) Printed Name: ---------- Site Name: ----------

APPENDIX K: SCHEDULE OF ASSESSMENTS

Table K-1: Required Assessments for T-Guard Arm

Assessment	Prior to Random-ization	Day of Randomization and Prior to Treatment	First Infusion	Second Infusion	Third Infusion	Fourth Infusion	Follow-up									
Target study day	-1	0	0	2	4	6	14	21	**28***	35	42	49	56	70	90	180[1]
Window (days)	-3 to -1	0	0 to 3	2 post-previous infusion	2 post-previous infusion	2 post-previous infusion	+/-3	+/-3	**+/-2**	+/-3	+/-3	+/-3	+/-3	+/-7	+/-7	+/-7
Study drug administration			X	X	X	X										
Acute GVHD evaluation, including bilirubin	X	X	X[2]	X	X	X	X	X	X	X	X	X	X	X[3]	X[3]	X[3]
Chronic GVHD evaluation									X				X		X	X
Medical History	X															
Physical exam (weight and height)		X														
Vital signs (temperature, pulse, respiratory rate)[4]			X	X	X	X										
Blood pressure			X	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC with differential and platelet count	X	X	X[2]	X	X	X	X	X	X				X		X	X
Albumin	X		X	X	X	X			X				X		X	X
Renal function (blood urea and/or blood urea nitrogen, Creatinine)	X					X	X	X	X							
Schistocytes, haptoglobin and LDH	X					X	X	X	X							
CK levels[5]	X															
Peripheral blood for CMV and EBV viral load		X				X	X	X	X	X	X	X	X			X
Pregnancy test (if applicable)[6]	X															
Karnofsky performance status		X							X				X		X	X
Pharmacokinetics[7, 8]			X	X	X	X										
Peripheral blood for Anti-Drug Antibody[7, 9]		X				X	X		X						X	X

Assessment	Prior to Random-ization	Day of Randomization and Prior to Treatment	First Infusion	Second Infusion	Third Infusion	Fourth Infusion	Follow-up									
Target study day	-1	0	0	2	4	6	14	21	**28***	35	42	49	56	70	90	180[1]
Window (days)	-3 to -1	0	0 to 3	2 post-previous infusion	2 post-previous infusion	2 post-previous infusion	+/-3	+/-3	**+/-2**	+/-3	+/-3	+/-3	+/-3	+/-7	+/-7	+/-7
Peripheral blood for GVHD biomarkers REG3α and ST2[7]		X				X	X		X							
Peripheral blood mononuclear cells (PBMC)[7]		X					X		X				X			X
Peripheral blood for immunophenotyping[7]		X					X		X				X			X
Urine sample for GVHD biomarker 3-IS[7]		X				X	X		X							
Concomitant medication review[10]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AE/SAE assessment[11]		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]Participants will be followed for survival post Day 180
[2]Assessment does not need to be repeated if treatment starts on same day as randomization
[3]Patients will be instructed to contact the site when their aGVHD symptoms worsen in between visits Day 70, 90 and 180
[4]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.
[5]If patients experience muscle pain, then a serum CK should be performed
[6]May be performed within 30 days of enrollment
[7]Research-specific samples must be drawn after participant signs consent, see APPENDIX B for more details
[8]Pharmacokinetic blood samples for T-Guard participants only. Samples directly at pre-infusion and at 4, 5, 6, 8, and 24 hours after start of 1st infusion; pre-infusion and at 4 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. A window of +/- 15 minutes is allowed for samples drawn at 4 hours after the start of infusion, a window of +/-30 minutes for the sample drawn 5, 6, and 8 hours after the start of infusion, and a window of +/- 1 hour for samples drawn 24 and 48 hours after the start of infusion. Every effort should be made to collect PK samples at all timepoints; however, ***no*** PK sampling is required on weekend days.
[9]Blood sample for ADA assessment should be collected for participants in the T-Guard treatment arm only. Day 6 sample should be collected just prior to the 4th dose infusion.
[10]All concomitant medications other than immunosuppressant agents will be collected starting at 7 days prior to randomization through 30 days after last dose of T-Guard. For all immunosuppressant agents, the overall start and stop date will be captured. For agents where dosing is tapered in response to drug levels, the medication dosing will not require reporting. Dosing for corticosteroids will be captured for all dosing changes.
[11]AEs and SAEs will be reported as described in Section 4.4 Adverse Event Reporting
*In the case where T-Guard was started 2 or 3 days after randomization, the Day 28 visit should be scheduled on either Day 29 or 30. If this scheduling is not possible, the Day 28 post-randomization visit should still be completed within the allotted window and a separate visit should be arranged at Day 28 +/- 2days post T-Guard initiation to collect aGVHD and systemic immunosuppression assessments.

Table K-2: Required Assessments for Ruxolitinib Arm

Assessment	Prior to Random-ization	Day of Randomization and Prior to Treatment	Treatment and Follow-up											
Target study day	-1	0	0	6	14	21	28	35	42	49	56	70	90	180[1]
Window (days)	-3 to -1	0	0 to 3	+/-3	+/-3	+/-3	+/-2	+/-3	+/-3	+/-3	+/-7	+/-7	+/-7	+/-7
Study drug initiation[2]			X											
Acute GVHD evaluation, including bilirubin	X	X	X[3]	X	X	X	X	X	X	X	X	X[4]	X[4]	X[4]
Chronic GVHD evaluation							X				X		X	X
Medical History	X													
Physical exam (weight and height)		X												
Blood pressure			X	X	X	X	X	X	X	X	X	X	X	X
CBC with differential and platelet count	X	X	X[3]	X	X	X	X				X		X	X
Albumin	X		X	X			X				X		X	X
Renal function (blood urea and/or blood urea nitrogen, Creatinine)	X			X	X	X	X							
Schistocytes, haptoglobin and LDH	X			X	X	X	X							
CK levels[5]	X													
Peripheral blood for CMV and EBV viral load		X		X	X	X	X	X	X	X	X			X
Pregnancy test (if applicable)[6]	X													
Karnofsky performance status		X					X				X		X	X
Peripheral blood for GVHD biomarkers REG3α and ST2[7]		X		X	X		X							
Peripheral blood mononuclear cells (PBMC)[7]		X			X		X				X			X
Peripheral blood for immunophenotyping[7]		X			X		X				X			X
Urine sample for GVHD biomarker 3-IS[7]		X		X	X		X							
Concomitant medication review[8]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AE/SAE assessment[9]		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

Assessment	Prior to Random-ization	Day of Randomization and Prior to Treatment	Treatment and Follow-up											
Target study day	-1	0	0	6	14	21	28	35	42	49	56	70	90	180[1]
Window (days)	-3 to -1	0	0 to 3	+/-3	+/-3	+/-3	+/-2	+/-3	+/-3	+/-3	+/-7	+/-7	+/-7	+/-7
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]Participants will be followed for survival post Day 180
[2]Refer to the pharmacy manual for details of ruxolitinib schedule
[3]Assessment does not need to be repeated if treatment starts on same day as randomization
[4]Patients will be instructed to contact the site when their aGVHD symptoms worsen in between visits Day 70, 90 and 180
[5]If patients experience muscle pain, then a serum CK should be performed
[6]May be performed within 30 days of enrollment
[7]Research-specific samples must be drawn after participant signs consent, see APPENDIX B for more details
[8]All concomitant medications other than immunosuppressant agents will be collected starting at 7 days prior to randomization through Day 44. For all immunosuppressant agents, the overall start and stop date will be captured. For agents where dosing is tapered in response to drug levels, the medication dosing will not require reporting. Dosing for corticosteroids will be captured for all dosing changes.
[9]AEs and SAEs will be reported as described in Section 4.4 Adverse Event Reporting