CTN 0301 trial for unrelated donor HSCT in SAA shows that of the 4 cyclophosphamide dose levels tested, 50 and 100 mg/kg in combination with ATG, fludarabine, and TBI (200 cGy) lead to better outcomes than the 0 mg/kg or 150 mg/kg dose.[12] Additionally, it is well-recognized that bone marrow (BM) is the preferred source of stem cells over peripheral blood stem cells (PBSC) in the unrelated donor setting, similar to MSD HSCT for SAA.[18-20]

Unfortunately, fully matched unrelated donors (HLA-A, B, C, DRB1) from worldwide registries can be identified for about 80% of Caucasians and for much lower percentages of persons of other races and/or ethnicities.[21] Outcomes using unrelated adult donors mismatched at a single HLA-locus (7/8 HLA-matched) have proven inferior to outcomes with fully matched unrelated donors (MUD), though allowing a single HLA-locus mismatched transplant greatly expands donor availability.[21, 22] With a sizable population of SAA patients who could benefit from unrelated donor HSCT who do not have fully matched donors, the need for improved alternative donor approaches has been identified as a key priority by an international working group on SAA convened by the BMT CTN in 2010.[23]

Registry data for alternative donor HSCT for SAA shows inadequate outcomes. Unpublished data from the CIBMTR for transplants performed between 2009 and 2013 in the US are summarized in Table 1 and show overall survival for each type of alternative donor transplant utilized for patients with SAA. The following sections will show more promising data associated with specific approaches to haplo HSCT that inform our proposal.

Table 1: Overall Survival in SAA by Donor Source

MUD BM 1-year OS	MUD PBSC 1-year OS	MMURD 1-year OS	UCB 1-year OS	Haplo*
Number=260 85% (95% CI 80-89%)	Number=72 72% (95% CI 61-82%)	Number=121 66% (95% CI 56-74%)	Number=45 58% (95% CI 43-72%)	Number=19 12 alive (12 of 19; 63%)

MUD = matched unrelated donor, MMURD = mismatched unrelated donor, MMRD = mismatched related donor (1 HLA-locus), Haplo = Haplo, UCB = unrelated cord blood, BM = bone marrow, PBSC = peripheral blood stem cells, OS = overall survival (95% CI = 95% confidence interval)
*Too few patients to calculate 1-year overall survival using the Kaplan-Meier estimator

1.2.2 **Haplo Donor Transplants in SAA**

The use of haplo donors for SAA transplantation has only more recently been attempted and accordingly there are only limited published data regarding outcomes with this approach. In small case series, rejection has been between 6% and 25%, acute GVHD between 12% and 30%, chronic GVHD between 20% and 40%, and overall survival between 62.5% and 84.6%.[24-26]

We pooled newer, unpublished data from Brazil, Johns Hopkins, and the Fred Hutchinson Cancer Research Center using a reduced intensity regimen with post-transplant cyclophosphamide in 29 patients. Results are promising and are shown below. All patients received a uniform conditioning regimen of TBI 200, fludarabine 150 mg/m^2 and Cy 29 mg/kg. Ten patients received ATG. Patients received Cy 100 mg/kg post-transplant in addition to methotrexate or calcineurin inhibitors for GVHD prophylaxis. There were 4 graft failures and 6

a. Bone marrow cellularity < 25% **or** marrow cellularity < 50% but with < 30% residual hematopoietic cells.

b. Two out of three of the following (in peripheral blood):

i. Neutrophils < 0.5 $x10^9$/L

ii. Platelets < 20 $x10^9$/L

iii. Reticulocyte count < 20 $x10^9$/L (< 60 x 10^9/L using an automated analysis)

3. No suitable fully matched related sibling donor (6/6 match for HLA-A and B at intermediate or high resolution and DRB1 at high resolution using DNA-based typing) available.

4. Failed at least one trial of immunosuppressive therapy (IST) by being refractory (persistence of severe cytopenias and fulfillment of SAA disease criteria at least 3 months after initial IST)[41] or having relapsed (initial improvement of cytopenias after first-line IST but then a later return to fulfillment of SAA disease criteria when IST is decreased or ceased)[41]. IST could have included ATG based regimens, calcineurin inhibitors and/or other higher dose therapy directed at the treatment of primary SAA.

5. Available relative of the patient who is a haploidentical match, including biological parents, siblings or half siblings, children, uncles/aunts, first cousins, etc. Eligible haploidentical donors will have 2-4 mismatches if HLA-A, -B, -C, and -DRB1 typing is used; 2-5 mismatches if HLA-A, -B, -C, -DRB1, and -DQB1 typing is used; and 2-6 mismatches if HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 typing is used. A unidirectional mismatch in either the graft versus host or host versus graft direction is considered a mismatch. The donor and recipient must demonstrate that they are a full haplotype match by being identical at a minimum of one allele (at high resolution DNA-based typing) at the following genetic loci: HLA-A, -B, -C, and DRB1 if 8 allele typing is used; HLA-A, -B, -C, -DRB1, and -DQB1 if 10 allele typing is used; and HLA-A, -B, -C, -DRB1-, DQB1, and -DPB1 is 12 allele typing is used by the local center. See Section 2.4 for additional information.

6. Patient and/or legal guardian must sign informed consent for HSCT.

7. The haplo donor and/or legal guardian must be able to sign informed consent documents.

8. The potential haplo donor must be willing and able to donate bone marrow.

9. The weight of the haplo donor must be ≥ 20 kg.

10. Adequate organ function defined as:

a. Cardiac: Left ventricular ejection fraction (LVEF) at rest ≥ 40%. For patients aged < 13 years, shortening fraction (SF) ≥ 26% by echocardiogram or MUGA may be substituted for LVEF.

b. Hepatic: Total bilirubin < 3.0 x the upper limit of normal (ULN) for age (patients who have been diagnosed with Gilbert's Disease are allowed to exceed this limit) and AST and ALT < 5.0 x ULN for age.

c. Renal:

PedsQL: The PedsQL™ Stem Cell Transplant Module is a 46-item instrument that measures HR-QoL in children and adolescents undergoing hematopoietic stem cell transplant, and is developmentally appropriate for self-report in ages 8 through 18 years.

2.5.5.2 **Administration**

The self-report questionnaires will be completed at Baseline, then at Day 100, Day 180, and Day 365 post-transplant or until death. Only English- and Spanish-speaking adult patients and English-speaking pediatric patients are eligible to participate in the HR-QoL component of this trial. Patients >18 years will complete the MOS SF-36. Patients 8 years through 18 years will complete the PedsQL™ Stem Cell Transplant Module. Surveys are completed by participants using self-completed instruments as a first choice. If this method of data collection is not possible, then surveys and response options may be read verbatim to participants, either in person or over the phone, to collect data. The method of survey completion, the date, and the language will be recorded in the database. **Surveys may not be completed by surrogates**.

Table 2.2 – Required Patient-Reported Outcomes Data Collection

Instrument	Number of Items	Baseline	Day 100	Day 180	Day 365
MOS SF-36	36	X	X	X	X
PedsQL™ SCT Module	46	X	X	X	X

decline in ANC to less than 0.5 x 10^9/L for three consecutive measurements on different days;
2. Initial whole blood or marrow donor chimerism greater than or equal to 5%, but then declining to less than 5% on subsequent measurements;
3. Second infusion/transplant given for graft failure.

3.2.3.3 **Alive with Autologous Recovery**

Autologous recovery is defined as ANC > 0.5 x 10^9/L and transfusion independence but with < 5% donor chimerism (whole blood or marrow).

3.2.4 **GVHD**

Acute and chronic GVHD are graded according to the BMT CTN Manual of Procedures (MOP).

3.2.5 **Immune Reconstitution**

Quantitative assessments of peripheral blood CD4, CD19, and CD56 positive lymphocytes will be done through flow cytometric analysis at baseline, Day 100, Day 180, and Day 365 post-transplant.

3.2.6 **Infection**

All Grade 2 and 3 infections will be reported according to the BMT CTN MOP. CMV viremia and disease, EBV viremia, HHV-6, and PTLD will be collected as per section 2.5.4.2.

3.2.7 **Health Related Quality of Life (HR-QoL)**

HR-QoL will be measured at Baseline and then at Day 100, Day 180, and Day 365 post-transplant using two instruments: the MOS SF-36 for adult patients (> 18 years), and the PedsQL™ Stem Cell Transplant Module for pediatric patients (8 years through 18 years). The instruments will be scored according to the recommendations of the developers. See Section 2.5.6 for detailed descriptions of the instruments. The SF-36 will be summarized by the Physical Component Summary (PCS) and Mental Component Summary (MCS). HR-QoL will be described for each treatment arm over time. Only adult patients able to read and speak in English or Spanish and pediatric patients able to read and speak in English are eligible to participate in the HR-QoL component of this trial.

12 week	84 ± 3 days
13 week	91 ± 3 days
100 day	100 ± 3 days
6 month	180 ± 28 days
12 month	365 ± 45 days

4.2.2 **Case Report Forms**

Criteria for Forms Submission: Criteria for timeliness of submission for all study forms are detailed in the Forms Guide. Forms that are not entered into AdvantageEDC™ within the specified time will be considered delinquent. A missing form will continue to be requested either until the form is entered into AdvantageEDC ™ and integrated into the Data Coordinating Center's (DCC) master database, or until an exception is granted and entered into the Missing Form Exception File, as detailed in the Forms Guide.

Reporting Patient Deaths: Recipient death information must be entered into AdvantageEDC ™ within 24 business hours of knowledge of the patient's death. If the cause of death is unknown at that time, it does not need to be recorded at that time. However, once the cause of death is determined, the Death Form must be updated in AdvantageEDC ™.

CIBMTR Data Reporting: Centers participating in BMT CTN trials must register pre- and post-transplant outcomes on all consecutive hematopoietic stem cell transplants done at their institution during their time of participation to the Center for International Blood and Marrow Transplant Research (CIBMTR). Registration is done using procedures and forms of the Stem Cell Transplant Outcomes Database (SCTOD). (Note: Federal legislation requires submission of these forms for all US allotransplant recipients.) Enrollment on BMT CTN #1502 must be indicated on the SCTOD pre-transplant registration form. Additionally, CIBMTR pre- and post-transplant Report Forms must also be submitted for all patients enrolled on this trial. CIBMTR forms will be submitted directly to the CIBMTR at the times specified on the Form Submission Schedule. Patients not undergoing HCT are not required to have their information reported to the CIBMTR.

Weekly GVHD Monitoring: GVHD should be monitored in accordance with BMT CTN guidelines as specified in the Manual of Procedures. Patients should be assessed weekly until Day +100 post-transplant for GVHD. After Day +100, patients will be assessed at each follow-up visit through 12 months for the presence of GVHD. For scheduling, a target day range has been provided in Table 4.2.1.

4.2.3 **Adverse Event Reporting**

4.2.3.1 **Definitions**

Adverse Event: An adverse event (AE) is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or any procedure specified in the protocol, even if the event is not considered to be related to the study.

Expectedness: An adverse event can be Expected or Unexpected

- **Expected adverse events** are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered

expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.

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

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

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

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

4.2.3.2 **Required Adverse Event Reporting**

Adverse event reporting will be consistent with BMT CTN procedures (BMT CTN Administrative Manual of Procedures, Chapter 6). It is BMT CTN policy that AEs must be reported even if the investigator is unsure whether a relationship exists between the adverse event and the use of study treatment. Unexpected, serious adverse events (SAEs) will be reported through an expedited AE reporting system via AdvantageEDC. Unexpected, life-

threatening and fatal SAEs must be reported within 24 hours of knowledge of the event. All other unexpected SAEs must be reported within three business days of knowledge of the event. Events entered in AdvantageEDC will be reported using NCI's Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. Expected AEs will be reported using NCI's Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 at regular intervals as defined on the Form Submission Schedule. Any expected life-threatening SAE not collected on another study form must be reported through the expedited AE reporting system via AdvantageEDC.

The Data and Safety Monitoring Board will receive summary reports of all unexpected SAEs on a semi-annual basis.

4.2.4 Patient Evaluations

Table 4.2.4 summarizes patient clinical assessments over the course of the study.

4.2.4.1 *Pre-transplant evaluations*

The following observations must be performed within 30 days prior to enrollment (unless noted otherwise):

1. History, physical examination, height, and weight (weight within 7 days prior to planned ATG infusion to ascertain ATG dosing).
2. Karnofsky or Lansky performance status.
3. Hematopoietic cell transplantation- comorbidity index (HCT-CI)
4. CBC with differential and platelet count and chemistries (creatinine, bilirubin, alkaline phosphatase, AST, ALT, ferritin). Please note that the absolute lymphocyte count is required within 7 days prior to planned ATG infusion to ascertain ATG dosing for UCB transplant recipients.
5. HLA typing (patient and donor) and anti-donor antibody testing (if not already performed). HLA typing may be done more than 30 days prior to enrollment.
6. Infectious disease titer for human immunodeficiency virus (HIV) by local standard of care.
7. Hepatitis B and C determined by serology and/or NAT.
8. LVEF or, for patients aged < 13.0 years, shortening fraction measurement by echocardiogram or MUGA.
9. Pulmonary function testing: FEV1, FVC, and DLCO (corrected for Hb). For patients aged < 13.0 years and patients unable to perform PFTs due to age or developmental ability, pulse oximetry is an acceptable alternative.
10. Bone marrow aspirate/biopsy with cytogenetics within 60 days prior to enrollment.
11. Diepoxybutane (DEB) testing on peripheral blood or comparable testing on marrow for Fanconi Anemia (at any time prior to enrollment).
12. Pregnancy test for females of childbearing potential (testing per institutional practice).
13. Study-required blood samples as described in Appendix C (all patients):
    a. Thymoglobulin pharmacokinetics (5 time points, 5 mL each time):

i. Upon completion of 1st dose of ATG (within 60 minutes)
ii. Prior to infusion of 2nd dose (within 60 minutes)
iii. Upon completion of 2nd dose of ATG (within 60 minutes)
iv. Upon completion of 3rd dose of ATG (within 60 minutes)
v. Day 0 (any time prior to infusion of graft)

CBC including absolute lymphocyte count should also be collected on Day 0 (if possible) along with this PK sample and results documented in AdvantageEDC; the timing of the sample (d-m-y hh:mm) should also be documented

14. Optional blood samples as described in Appendix C:
    a. Telomere length assay (all patients and haplo donors; collected pre-conditioning for patients, pre-donation for donors)
        i. Children < 20 kg: 12 mL in EDTA tubes
        ii. Adults: 24 mL in EDTA tubes
    b. Future research (all patients): 3mL whole blood prior to conditioning
15. Absolute lymphocyte numbers by flow cytometry for lymphocyte subpopulations to include CD4, CD19, and CD56.
16. Serum quantification of IgG, IgM, and IgA.
17. Baseline peripheral blood samples for chimerism analysis by molecular methods (patient and donor).
18. Health Related Quality of Life (HR-QoL): Patient 'self-reported' assessments to include the Medical Outcomes Study Short Form 36 (MOS SF-36) for English and Spanish speaking adult patients (> 18 years). English-speaking pediatric patients (ages 8 through 18 years) will complete the PedsQL™ Stem Cell Transplant Module.

4.2.4.2 ***Post-transplant evaluations***

1. History and physical exam to assess GVHD and other morbidities weekly until Day +100, then at Day +180 and Day +365. GVHD evaluation and grading to be in keeping with the BMT CTN MOP. Chronic GVHD Provider Survey to be completed by an MD, NP, or PA at the time of patient assessment. For scheduling purposes, a target day range has been provided in Table 4.2.1.
2. Karnofsky or Lansky performance status at Day +365.
3. CBC at least three times a week (or as per institution's standard practice) from Day 0 until ANC > 0.5 x 10^9/L for 3 consecutive measurements over 3 or more days. Thereafter, CBC at least twice per week (or as per institution's standard practice) until Day +28, then weekly until Day +100, then at Day +180 and Day +365.
4. Chemistries (creatinine, bilirubin, alkaline phosphatase, AST, ALT) twice a week until Day +28 and then weekly until Day +100, then at Day +180 and Day +365 (or as per institution's standard practice). Cyclosporine or tacrolimus levels will be measured at

least once weekly until Day +100, and then at each follow-up visit until the drug is tapered off (or as per institution's standard practice).

5. Toxicity assessments at Day +28, +56, +100, +180, and +365.
6. Absolute lymphocyte numbers by flow cytometry for lymphocyte subpopulations to include at minimum CD4, CD19, and CD56 at Day +100, +180, and +365.
7. Serum quantification of IgG, IgM, and IgA at Day +365.
8. Quantification of peripheral blood (whole blood and CD3) or marrow chimerism (including lineage-specific, myeloid, and T cell chimerisms) at Day +28, Day +56, Day +100, Day +180, and Day +365. **In the event that Day 100 chimerism results at less than 50% donor in either myeloid or T cell compartment, additional chimerism testing of the same compartment should be performed every 4 weeks or as clinically indicated until the chimerism is greater than or equal to 50% donor and/or stabilizes.**
9. EBV DNA quantitative PCR testing and CMV DNA quantitative PCR testing on peripheral blood at least weekly until Day +100 and then per institutional practice. It is recommended to continue weekly or every other week until the patient is off of all immunosuppression.
10. Study-required blood samples as described in Appendix C (all patients):
    a. Thymoglobulin pharmacokinetics (1 time point, 5 mL):
        i. Day +7 (no time restrictions)
11. Health Related Quality of Life (HR-QoL): Patient 'self-reported' assessments at Day +100, Day +180, and Day +365 post-transplant to include the Medical Outcomes Study Short Form 36 (MOS SF-36) for English- and Spanish-speaking adult patients (> 18 years). English-speaking pediatric patients (ages 8 through 18 years) will complete the PedsQL™ Stem Cell Transplant Module.

5.3 Interim Analysis and Pausing Guidelines

There will be no interim analyses for efficacy or futility. Patients will be monitored for key safety endpoints including graft failure and mortality. Graft failure will be monitored including both primary graft failure and secondary graft failure. Primary graft failure will be determined by Day 56 post-transplant in all patients receiving a transplant. Secondary graft failure will be determined by Day 56 post-transplant in all patients achieving the initial engraftment. Mortality will be monitored up to 115 days from the first day of preparative regimen (Day -9) in all patients receiving the first dose of ATG. The rationale for monitoring mortality from the first day of ATG preparation in all patients receiving the first dose of ATG is to guard against excessive early mortality including death due to the intervention in patients who never proceed to transplant, although this event is expected to be extremely uncommon. The monitoring period for mortality is set to 115 days from the first day of ATG preparation to ensure that this time point aligns with the Day-100 post-transplant evaluation for patients receiving a transplant.

Graft failure and mortality will be monitored so that if rates significantly exceed pre-set thresholds, the NHLBI will be notified in order that the DSMB can be advised. Policies and composition of the DSMB are described in the BMT CTN's Manual of Procedures. The pausing guidelines serve as a trigger for consultation with the DSMB for additional review and are not formal “stopping rules” that would mandate automatic closure of study enrollment.

A truncated Sequential Probability Ratio Test (SPRT) based on a binomial test of proportions will be used to monitor each safety endpoint as described below. This sequential testing procedure conserves type I error across all of the monitoring looks for each endpoint, but not across endpoints. The SPRT can be represented graphically. At each interim analysis, the number of patients evaluable for the endpoint is plotted against the total number of patients who have experienced the event. The continuation region of the SPRT is defined by two parallel lines. Only the upper boundary will be used for monitoring to protect against excessive graft failure or mortality. If the graph falls above the upper boundary, the SPRT rejects the null hypothesis, and concludes that graft failure or mortality is higher than predicted by the number of patients on study. Otherwise, the SPRT continues until enrollment reaches the target goal.

The usual measures of performance of an SPRT are the error probabilities α and β of rejecting H_0 when $\theta = \theta_0$ and of accepting H_1 when $\theta = \theta_1$, respectively, and the expected sample size $E(N|\theta_i)$. Note that since the test uses only the upper boundary, and is truncated by a finite sample size, the size of the test will be slightly lower than the nominal level.

5.3.1 Graft Failure by Day 56 Post Transplant

Graft failure will be monitored up to Day 56 post-transplant in all patients who proceed to transplant. Events for this safety endpoint include both primary graft failure and secondary graft failure. The safety boundary for graft failure was developed from an SPRT contrasting 15% versus 35% graft failure by Day 56 post-transplant, with nominal type I and II errors of 10% and 14%, respectively. The slope of the line for monitoring excessive graft failure is 0.240 and the intercept is 1.929. The stopping rule is summarized in Table 5.3.1A.

Neutrophil Engraftment

Cumulative incidence of neutrophil engraftment at Day 28 will be estimated with a 95% confidence interval using the cumulative incidence function with death prior to neutrophil engraftment as the competing risk.

Platelet Engraftment

Cumulative incidence of platelet engraftment at Day 100 will be estimated with a 95% confidence interval using the cumulative incidence function with death prior to platelet engraftment as the competing risk.

Primary and Secondary Graft Failure

The frequency and proportion of patients experiencing primary graft failure by Day 56 and the proportion of patients who have engrafted and subsequently experience secondary graft failure will be described with 95% confidence interval.

Grade II – IV Acute GVHD

Cumulative incidence of Grade II – IV acute GVHD at Day 100 will be estimated with a 95% confidence interval using the cumulative incidence function with death prior to Grade II – IV acute GVHD as the competing risk.

Chronic GVHD

Cumulative incidence of chronic GVHD at one year will be estimated with a 95% confidence interval using the cumulative incidence function with death prior to chronic GVHD as the competing risk. This will include analysis of the severity of chronic GVHD using the NIH criteria.

Immunologic Reconstitution

Immune reconstitution assays including CD4, CD19, and CD56 will be measured pre-HCT and at Day 91, Day 180, and Day 365 post-HCT. These will be summarized at each time point using descriptive statistics.

Incidence of Infectious Complications

The number of specific infections (documented bacteremia with organism, CMV, and EBV) and the number of patients experiencing infections will be tabulated. Incidences of CMV viremia and disease, EBV viremia, and PTLD will be reported.

Health Related Quality of Life (HR-QoL)

HR-QoL will be measured at Baseline and then at Day 100, Day 180, and Day 365 post-transplant using two instruments: the MOS SF-36 (both PCS and MCS) for English- and Spanish-speaking adult patients (> 18 years) and the PedsQL^{TM} Stem Cell Transplant Module for English-speaking pediatric patients (8 years through 18 years). HR-QoL at each time point will be summarized using simple descriptive statistics (mean, SD). Analysis will be done separately for each instrument using a Bonferroni adjusted significance level (0.05/4). All models will be adjusted for baseline HR-QoL.

deaths. Twenty-three of 29 patients are alive with variable follow up: 10 patients were followed for less than 6 months after transplantation, 4 patients have been followed between 6 and 12 months, and 9 patients have been followed for longer than 12 months.

Table 2: Haplo Donor Transplants in SAA

Patient #	Conditioning	Engraftment	Last Follow-Up	Status
1	Cy29/Flu150/TBI200/ATG	Yes, 100% donor	36 months	Alive
2	Cy29/Flu150/TBI200/ATG	Yes, 100% donor	13 months	Alive
3	Cy29/Flu150/TBI200/ATG	Yes, 100% donor	18 months	Alive
4	Cy29/Flu150/TBI200/ATG	Yes, 100% donor	7 months	Alive
5	Cy29/Flu150/TBI200/ATG	Yes, 100% donor	17 months	Alive
6	Cy29/Flu150/TBI200/ATG	Yes, 100% donor	3 months	Alive
7	Cy29/Flu150/TBI200/ATG	Yes, 100% donor	44 months	Alive
8	Cy29/Flu150/TBI200/ATG	Yes, 100% donor	6 months	Alive
9	Cy29/Flu150/TBI200	Primary Graft Failure	2 months	Died
10	Cy29/Flu150/TBI200	Yes, 100% donor	28 months	Alive
11	Cy29/Flu150/TBI200	Yes, 100% donor	9 months	Alive
12	Cy29/Flu150/TBI200	Yes, 100% donor	24 months	Alive
13	Cy29/Flu150/TBI200	Yes, 100% donor	17 months	Alive
14	Cy29/Flu150/TBI200	Yes, 100% donor	11 months	Died
15	Cy29/Flu150/TBI200	Yes, 100% donor	6 months	Alive
16	Cy29/Flu150/TBI200	Yes, 100% donor	3 months	Alive
17	Cy29/Flu150/TBI200	Primary Graft Failure	4 months	Died
18	Cy29/Flu150/TBI200	Primary Graft Failure	1 month	Alive
19	Cy29/Flu150/TBI200	Yes, 100% donor	3 months	Died

i. For patients ≥ 13.0 years of age at the time of enrollment: estimated creatinine clearance > 50 mL/minute (using the Cockcroft-Gault formula[1] and actual body weight). Please refer to Body Weight calculations in Appendix E.

ii. For patients < 13.0 years of age at enrollment: GFR estimated by the updated Schwartz formula[2] ≥ 90 mL/min/1.73 m^2. If the estimated GFR is < 90 mL/min/1.73 m^2, then renal function must be measured by 24-hour creatinine clearance or nuclear GFR, and must be > 50 mL/min/1.73 m^2.

d. Pulmonary:

i. For patients ≥ 13.0 years of age: DLCO (corrected/adjusted for hemoglobin) > 40% **and** FEV1 > 50% predicted (without administration of bronchodilator) **and** FVC > 50% predicted.

ii. For patients < 13.0 years of age unable to perform PFTs due to age or developmental ability: (1) no evidence of dyspnea at rest **and** (2) no need for supplemental oxygen **and** (3) O2 saturation > 92% on room air at sea level (with lower levels allowed at higher elevations per established center standard of care (e.g., Utah, 4,200 feet above sea level, does not give supplemental oxygen unless below 90%)).

11. Karnofsky or Lansky performance status ≥ 60%.
12. Females and males of childbearing potential must agree to practice 2 effective methods of contraception at the same time or agree to abstinence.

2.3.2 Patient Exclusion Criteria

1. Inherited bone marrow failure syndromes such as Fanconi anemia must be ruled out according to center standards.
2. Clonal cytogenetic abnormalities consistent with pre-myelodysplastic syndrome (pre-MDS) or MDS on marrow examination (e.g. Monosomy 7).
3. Diagnosis of myelodysplastic syndrome (MDS).
4. Presence of anti-donor HLA antibodies (positive anti-donor HLA antibody is defined as a positive cross-match test of any titer by complement-dependent cytotoxicity or flow cytometric testing or the presence of anti-donor HLA antibody to the high expression loci HLA-A, B, C, DRB1, or DPB1 with mean fluorescence intensity (MFI) > 1000 by solid phase immunoassay).
5. Prior allogeneic stem cell transplant.

[1] Cockcroft-Gault formula, based on ideal body weight (IBW): $CrCl = \frac{(140 - age) \times IBW\ (kg)}{P_{Cr} \times 72} \times 0.85$ for females

[2] Schwartz equation: CrCl (mL/min/1.73m^2) = [length (cm) x k] / serum creatinine
k = 0.45 for infants 1 to 52 weeks old
k = 0.55 for children 1 to 13 years old
k = 0.55 for adolescent females 13-18 years old
k = 0.7 for adolescent males 13-18 years old

CHAPTER 3

3 STUDY ENDPOINTS AND DEFINITIONS

3.1 Primary Endpoint

3.1.1 Overall Survival (OS)

The primary endpoint is overall survival (OS) at 1 year post-HSCT in patients with SAA. OS is defined as the time interval from date of transplant to death or to last follow-up, whichever occurs first.

3.2 Secondary Endpoints

3.2.1 Neutrophil Recovery

Neutrophil recovery is achieving an ANC > 0.5 $x10^9$/L for three consecutive measurements on different days, with the first of the three days being defined as the day of neutrophil engraftment.

3.2.2 Platelet Recovery

Platelet recovery is defined by achieving a platelet count > 20 x 10^9/L with no platelet transfusions in the preceding seven days. The first day of the sustained platelet count will be defined as the day of platelet engraftment.

3.2.3 Alive with Sustained Engraftment

Being alive and engrafted is defined as not having experienced death, primary graft failure, or secondary graft failure.

Donor cell engraftment is defined as donor chimerism greater than or equal to 5% on or after Day 56 after transplantation. Chimerism may be evaluated in whole blood or blood cell fractions, including CD3 and CD33 or CD15 fractions. For this protocol, lineage-specific, myeloid, and T cell chimerisms are required.

3.2.3.1 Primary Graft Failure

Primary graft failure is defined by the lack of neutrophil engraftment by Day +56 post-HSCT or failure to achieve at least 5% donor chimerism (whole blood or marrow) on any measurement up to and including Day +56.

For this protocol, lineage-specific, myeloid, and T cell chimerisms are required. Myeloid engraftment might not proceed at the same rate as T cell engraftment. If myeloid has greater than or equal to 5% donor, even if T cell compartment does not, this is not considered primary graft failure.

3.2.3.2 Secondary Graft Failure

Secondary graft failure is defined as any one of the following:

1. Initial neutrophil engraftment (ANC greater than or equal to 0.5 $x10^9$/L measured for three consecutive measurements on different days) followed by sustained subsequent

CHAPTER 4

4 PATIENT ENROLLMENT AND EVALUATION

4.1 Enrollment Procedures

Patients will be registered using the BMT CTN Electronic Data Capture System (AdvantageEDC™). The following procedures should be followed:

1. Prior to initiation of the conditioning regimen, an authorized user at the transplant center completes the Demographics Form and Segment 0 Enrollment Form in AdvantageEDC, at which point a study number will be generated. After patient is enrolled on Segment 0, the authorized user should complete the HLA Forms to verify an eligible HLA match score. This eligibility screening includes a question confirming that the patient (or legally authorized representative) signed informed consent. The eligibility screening also includes a question confirming that the donor (or legal guardian) signed informed consent, agrees to provide marrow, and weighs at least 20 kg.
2. After filling out the HLA Forms, the authorized user will be able to enroll the patient on Segment A using the Segment A Enrollment Form. **The patient is not officially enrolled on study and may not begin study treatment until they are enrolled on Segment A.**
3. A visit schedule based on transplant date is displayed for printing and is referred to as "Segment A Follow-up".

4.2 Study Monitoring

4.2.1 Follow-up Schedule

The follow-up schedule for study visits is outlined in Table 4.2.1. A detailed description of each of the forms and the procedures required for forms completion and submission can be found in the BMT CTN 1502 Forms Guide.

Table 4.2.1: Follow-Up Schedule

Study Visit	Target Day Post-Transplant
1 week	7 ± 3 days
2 week	14 ± 3 days
3 week	21 ± 3 days
4 week	28 ± 3 days
5 week	35 ± 3 days
6 week	42 ± 3 days
7 week	49 ± 3 days
8 week	56 ± 3 days
9 week	63 ± 3 days
10 week	70 ± 3 days
11 week	77 ± 3 days

Table 4.2.4: Summary of Assessments

Study Assessments / Testing	Baseline (Pre-conditioning)	During conditi-oning	0	7	14	21	28	35	42	49	56	63	70	77	84	91	100	180	365
History, Physical Exam, Weight, and Height	X																		
Karnofsky/Lansky Performance Status	X																		X
CBC[1], Differential, Platelet Count, and Blood Chemistries[2]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HLA Typing & Anti-Donor Antibody Testing	X																		
Infectious Disease Titers[3]	X																		
LVEF or Shortening Fraction for < 13 years	X																		
Pulmonary Function Tests[4]	X																		
Bone Marrow Aspirate for Pathology and Cytogenetics[5]	X																		
Testing for Fanconi Anemia[6]	X																		
Pregnancy Test (females only)	X																		
Study-required Blood Samples[7]		X	X	X															
Optional Blood Samples[8]	X																		
CD4, CD19, and CD56 Counts	X																X	X	X
Serum Quantification of IgG, IgM, and IgA	X																		X
Peripheral Blood Chimerism[9]	X						X				X						X	X	X
CMV & EBV DNA Quantitative PCR Testing[10]				X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Toxicity Assessments							X				X						X	X	X
Acute GVHD				X	X	X	X	X	X	X	X	X	X	X	X	X			
Chronic GVHD (including Provider surveys)[11]																	X	X	X
Comprehensive CIBMTR Forms & HR-QoL	X																X	X	X

[1] CBC performed three times weekly from Day 0 until ANC > 0.5 x 10^9/L for three consecutive measurements on different days. CBC then performed twice per week until Day +28, then weekly until Day +100, then at Day +180 and Day +365. CBC including absolute lymphocyte count should be collected on Day 0 along with the PK sample (if possible).

[2] Blood chemistries include: creatinine, bilirubin, alkaline phosphatase, AST, ALT, Ferritin (baseline only) and cyclosporine or tacrolimus level. Cyclosporine or tacrolimus levels will be measured at least once weekly until Day +100 and then at each follow-up visit until tapered off. Blood chemistries performed twice weekly until Day +28 and then weekly until Day +100, then at Day +180 and Day +365.

[3] Infectious disease titers include: hepatitis panel (HepB SAb, HepB SAg, HepB Core Ab, HepC Ab) and HIV.

[4] DLCO (corrected for Hb), FEV1, and FVC or pulse oximetry for patients aged < 13.0 years if unable to perform standard PFT.

[5] Bone marrow aspirate and biopsy (cytogenetics is required, local MDS panel recommended) within 60 days prior to enrollment.

[6] Results of Diepoxybutane (DEB) testing on peripheral blood or comparable testing on marrow for Fanconi anemia at any time prior to enrollment.

[7] Study-required blood draws include 6 samples for Thymoglobulin® pharmacokinetics in all patients (4 samples during ATG dose days, 1 sample on Day 0, and 1 sample on Day +7).

[8] Optional blood draws include a baseline sample for telomere length assay collected from all consenting patients pre-conditioning and consenting haplo donors pre-donation as well as 1 sample for future research collected from all consenting patients pre-conditioning.

[9] Chimerism to be measured by standard molecular testing of a peripheral blood (whole blood and CD3) sample or marrow chimerism (including lineage-specific, myeloid, and T cell chimerisms).

[10] EBV and CMV monitoring is required weekly until Day +100, and then per institutional practices. It is recommended to continue monitoring weekly or every other week until off all immunosuppression.

[11] Chronic GVHD Provider Survey to be completed by an MD, NP, or PA at the time of patient assessment.

TABLE 5.3.1A PAUSING GUIDELINES FOR DAY-56 GRAFT FAILURE

Number of Patients Proceed to Transplant	Pause if Graft Failure Occurs in
3 – 4	3
5 – 8	4
9 – 12	5
13 – 16	6
17 – 21	7
22 – 25	8
26 – 29	9
30	10

The actual operating characteristics of the truncated test, shown in Table 5.3.1B, were determined in a simulation study with 10,000 replications. The simulation assumed uniform accrual of 30 patients over a three-year time period.

TABLE 5.3.1B OPERATING CHARACTERISTICS OF SEQUENTIAL TESTING PROCEDURE FOR DAY-56 GRAFT FAILURE FROM A SIMULATION STUDY WITH 10,000 REPLICATIONS

True Day-56 rate	15%	20%	25%	30%	35%
Probability reject the null hypothesis	0.07	0.20	0.41	0.63	0.80
Mean month stopped	36.6	34.1	30.2	25.6	21.0
Mean # endpoints in 56 days	4.4	5.4	6.0	6.0	5.7
Mean patients evaluable for endpoints	28.9	26.9	23.7	20.0	16.3

Graft failure will be monitored in all patients who actually receive a BM infusion. The SPRT rejects the null hypothesis in favor of the alternative 7% of the time when the true Day-56 rejection rate is 15%, and 80% of the time when the true Day-56 rejection rate is 35%. This corresponds to a type I error rate $\alpha = 0.07$ and a type II error rate $\beta = 0.20$. When the true Day-56 rejection rate is 35%, on average, the DSMB will be consulted 21 months after opening, when 6 events have been observed in 17 patients. Note that the SPRT procedure is adequately powered to distinguish between a rejection rate of 15% and 35%.

5.3.2 Mortality by Day 115 Post ATG Preparation

Mortality will be monitored up to 115 days from the first day of ATG preparation (~100 days post-transplant for patients receiving a transplant). Mortality will be monitored in all patients receiving the first dose of ATG. The safety boundary for mortality was developed from an SPRT contrasting 10% versus 30% mortality rate, with nominal type I and type II errors of 8% and 15%, respectively. The slope of the line for monitoring excessive mortality is 0.186 and the intercept is 1.751. The stopping rule is summarized in Table 5.3.2A.

TABLE 5.3.2A PAUSING GUIDELINES FOR 115 DAY MORTALITY

Number of Patients Started ATG Preparation and Evaluable for Mortality by 115 Days	Pause if Mortality Occurs in
3 – 6	3
7 – 12	4

APPENDIX A:

HUMAN SUBJECTS